ADVANCES IN AGRONOMY



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ADVANCES IN AGRONOMY

Edited by

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PREFACE

Chapter 118 contains seven comprehensive reviews on contemporary topics in the crop and soil sciences. Chapter 1 is a review dealing with digital mapping of carbon, an element of global significance. Chapter 2 assesses the impacts of climate change and variability on seed production and the seed industry. Chapter 3 provides a thorough review of competitive sorption mechanisms of ions at the mineral/water interface. Chapter 4 is a timely review on the soybean genome. Chapter 5 covers crop responses to ammonium and nitrate. Chapter 6 provides insights on flaming as an approach to control weeds in agronomic crop systems. Chapter 7 discusses the role that ridge-furrow mulching systems can play in sustaining agriculture in semiarid environments.

I appreciate the authors' outstanding reviews.

Donald L. Sparks
Newark, Delaware, USA



CHAPTER ONE

Digital Mapping of Soil Carbon

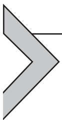
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Contents

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Abstract

There is a global demand for soil data and information for food security and global environmental management. There is also great interest in recognizing the soil system as a significant terrestrial sink of carbon. The reliable assessment of soil carbon (C) stocks is of key importance for soil conservation and in mitigation strategies for increased atmospheric carbon. In this article, we review and discuss the recent advances in digital mapping of soil C. The challenge to map carbon is demonstrated with the large variation of soil C concentration at a field, continental, and global scale. This article reviews recent studies in mapping soil C using digital soil mapping approaches. The general activities in digital soil mapping involve collection of a database of soil carbon observations over the area of interest; compilation of relevant covariates (*scorpan* factors) for the area; calibration or training of a spatial prediction function based on the observed dataset; interpolation and/or extrapolation of the prediction function over the whole area; and finally validation using existing or independent datasets. We discuss several relevant aspects in digital mapping: carbon concentration and carbon density, source of data, sampling density and resolution, depth of investigation, map validation, map uncertainty, and environmental covariates. We demonstrate harmonization of soil depths using the equal-area spline and the use of a material coordinate system to take into consideration the varying bulk density due to management practices. Soil C mapping has evolved from 2-D mapping of soil C stock at particular depth ranges to a semi-3-D soil map allowing the estimation of continuous soil C concentration or density with depth. This review then discusses the dynamics of soil C and the consequences for prediction and mapping of soil C change. Finally, we illustrate the prediction of soil carbon change using a semidynamic *scorpan* approach.



1. INTRODUCTION

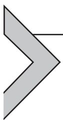
Soil carbon (C) is recognized as the largest store of terrestrial carbon (Batjes, 1996; Jobbagy and Jackson, 2000; Lal, 2004). Globally, its storage capacity is much larger compared with the pools of carbon in the atmosphere and vegetation. There is now a large and growing interest in knowing the size of soil carbon pool and its sequestration potential. Mapping the spatial distribution of soil carbon has been of great interest as exemplified by the increasing number of publications in mapping soil carbon stock globally and nationally (Grunwald, 2009). This is reflecting the response to the demand for a more accurate assessment of soil carbon pool at a better resolution. Many articles have been published, quantifying and mapping soil carbon storage at the field, landscape, regional, continental, and global scales (Bernoux et al., 2002; Post et al., 1982). Conventional methods that used soil maps as the basis of soil carbon estimates are still being used for mapping areas that have a limited number of soil observations (Batjes, 2008b). However, digital soil mapping technology has progressed rapidly in the past

decade, making it operational for routine mapping over large areas (Bui et al., 2009; Grunwald et al., 2011; Rawlins et al., 2009; Triantafilis and Buchanan, 2010). Digital soil mapping was identified as one of the emerging research fronts in agricultural sciences in the December 2009 issue of the Thompson Reuters Essential Science Indicators[SM1]. Polygon-based soil maps are now being replaced with digital maps of soil carbon content and their associated uncertainties for new areas or previously mapped areas. These maps are stored and manipulated in digital form within a Geographical Information System (GIS) environment, creating the possibility of vast arrays of data for analysis and interpretation (Grunwald, 2009; Meersmans et al., 2009; Mueller and Pierce, 2003; Triantafilis et al., 2009).

This article will review the state of the art in mapping soil carbon and soil carbon change by using digital soil mapping approaches. Mapping and the knowledge of the spatial distribution of soil carbon is useful to

- Provide a baseline carbon level, which can be useful when soil carbon is included in greenhouse gas emissions trading schemes;
- Help localize the variables controlling soil carbon;
- Assist in natural resource management and monitoring;
- Identify potential project locations for soil-based carbon sequestration; and
- Serve as an input into mechanistic simulation models.

There is, in general principles, an essential difference between mapping of soil carbon and accounting of soil carbon. Mapping activity attempts to give an image of the spatial distribution of soil carbon, and while we can use mapping for temporal soil carbon auditing, it will generally be an expensive exercise. In auditing, we are only interested in knowing the total amount of carbon over an area for a particular depth at a particular time, and we do not need to know the exact spatial distribution of carbon. The efficiency of auditing is in the use of statistically design-based sampling strategy (Brus and de Gruijter, 2011). As it is a substantial topic of its own, the issue of auditing will not be discussed here.



2. REVIEW OF PAST STUDIES

2.1. Past Studies

There have been numerous global estimations of soil carbon stocks, and most of them are derived from existing soil maps. The results vary and do

[1] http://sciencewatch.com/dr/erf/2009/09decerf/.

not state the uncertainty of estimates, for example, the reported estimates for global soil organic carbon (SOC) pool in the upper 1-m profile vary from 1220 Pg (Sombroek et al., 1993), 1395 Pg (Post et al., 1982), 1456 Pg (Schlesinger, 1977), 1462–1548 Pg (Batjes, 1996), 1502 Pg (Jobbagy and Jackson, 2000), and 1550 Pg (Lal, 2004). These variable results could be due to the effect of different methods used and also to the variability in spatial and temporal status of the data.

Conventional methods are still being used for the estimation of soil carbon stock for a region or continent; the estimates are based on existing soil maps using soil–landscape and vegetation associations. The resulting maps are usually in the cartographic scale of 1:1,000,000 or coarser, for example, Africa (Henry et al., 2009), Central Africa (Batjes, 2008b), Brazil (Bernoux et al., 2002), and Congo (Schwartz and Namri, 2002). These maps are indeed still useful where there is little soil information for the area. These maps were used by Milne et al. (2007) in the Global Environment Facility Soil Organic Carbon modeling system to map future SOC stock changes in Brazilian Amazon (Cerri et al., 2007), the Indo-Gangetic plains (Bhattacharyya et al., 2007), and Jordan (Al-Adamat et al., 2007).

Since the development of digital soil mapping technologies in the late 1990s, and formalization of the discipline by McBratney et al. (2003), mapping of soil carbon at the field and regional scales has become an area of active research. Table 1.1 summarizes some recent studies of soil carbon concentration and carbon density maps that have been produced using digital soil mapping technology with the *scorpan* model. Here, we only list studies that have used the *scorpan* approach.

The approach of digital soil mapping follows the *scorpan* spatial prediction function:

$$C_x = f(s, c, o, r, p, a, n) + e, \quad (1)$$

where soil carbon C at spatial position x is a function of soil factors (s), climate (c), organisms, which include land use, human effects, and management (o), relief (r), parent materials (p), age or time (a), spatial position (n), and e is the spatially correlated errors. Except for the "time" or "age" factor, most digital soil mapping examples have either explicitly or implicitly used these factors for prediction of soil carbon. However "time" is also an essential factor in soil carbon prediction. Soil carbon observations denoted as "*s*" on the right-hand side of the equation are

required to calibrate this model. The assumption is also that the observation should cover the whole range variation in covariates, so that the model can be extrapolated to the whole area. The form of *f* can be a simple linear model to more complicated data-mining tools such as regression trees and random forests (Table 1.1).

2.2. What Do We Learn from These Studies?

The activities conducted by most studies (Table 1.1) are as follows: (i) collection of a database of soil carbon observations over an area of interest; (ii) compilation of relevant covariates for the area; (ii) calibration or training of a spatial prediction function based on the observation dataset; (iii) interpolation and/or extrapolation of the prediction function over the whole area; and (iv) validation based on existing or independent datasets.

A summary of studies cited in Table 1.1 is as follows:

2.2.1. Sources of Data

For field and watershed scale studies, most studies collected soil samples that were guided by environmental covariates. For regional and continental studies, except for France, UK, or nations that have a national monitoring network, most studies were based on legacy soil data.

2.2.2. Extent, Resolution, and Sample Density

Soil carbon has been mapped using digital soil mapping technology at field, regional, national and continental scales with a sampling density from 0.002 to 1100 samples per km^2. Figure 1.1 shows that generally the grid spacing (resolution) of the digital maps increases logarithmically with extent, and the grid spacing decreases logarithmically with sampling density. Although there is no general rule for sample density and grid spacing in digital soil mapping, it also does not mean that we can confidently generate maps at a high resolution using low sampling densities. The uncertainty of prediction should reflect this. Although there are large variations, involving various studies at different depths, the graph shows that the prediction accuracy increases logarithmically with increasing density of observation (Fig. 1.1c).

2.2.3. Depth

Most studies predict soil carbon stock for the top 10–30 cm, and only a few studies have measured carbon stock down to 1 m.

Table 1.1 A review of recent studies on digital mapping of soil carbon

Study area	Extent (km^2)	Maximum depth of prediction (cm)	Grid spacing/ resolution	Number of samples	R^2 prediction	Validation	Covariates	Fitting methods	References
Australia	2,765,000	30	250	11,483	0.41	External	Climate, elevation, lithology, moisture index, soil class	Piecewise linear decision tree	(Bui et al., 2009)
Midwest USA	658,168	50	30	2103		Internal	Terrain attributes, climate, land cover, geology, MODIS NDVI	Geographically weighted regression	(Mishra et al., 2010)
France	543,965	30	12,000	2200	0.91	Internal	Climatic parameters, vegetation NPP, soil properties, and land use	Boosted regression tree	(Martin et al., 2011)
Laos	230,566	100	5	2806	0.42	Internal	Relief, climate, soil map	Cokriging	(Phachomphon et al., 2010)
Agricultural areas, NSW, Australia	158,000	100	250	1145	0.57	Internal	Terrain attributes, climate, land cover, lithology, gamma radiometrics	Piecewise linear decision tree	(Wheeler et al., in press)

Hebei province, China	187,693	100	100	359	0.6	Internal	Terrain attributes, AVHRR NDVI	ANN Regression kriging	(Zhao and Shi, 2010)
Ireland	71,000	10	500	1310		Internal	Rainfall, land cover, soil type	Geographically weighted regression	(Zhang et al., 2011)
Rio de Janeiro State, Brazil	44,000	10	90	431		No	Terrain attributes, Landsat, land cover, lithology	Regression kriging	(Mendonça Santos et al., 2010)
England	18,165	Topsoils	500	5678		No	-	Ordinary kriging	(Rawlins et al., 2011)
Northern Ireland	13,550	20	50	6862		Internal	Gamma K, elevation, soil type	Linear mixed model	(Rawlins et al., 2009)
Southeastern Kenya	13,500	30	1000	95	0.21	Crossvalidation	Climate, topography, vegetation	Regression kriging	(Stoorvogel et al., 2009)
Northern Italy	12,000	30	1000	18,969	0.82	Internal	Soil maps	Regression kriging	(Ungaro et al., 2010)
Flanders, Belgium	10,179	100	15	6900	0.36	No	Land use, soil type, depth to groundwater	Linear model	(Meersmans et al., 2008)
Denmark	5748	20	50	19,836		Internal	Parent material, soil type, topography, NDVI	Classification tree	(Bou Kheir et al., 2010)

Continued

Table 1.1 A review of recent studies on digital mapping of soil carbon—cont'd

Study area	Extent (km^2)	Maximum depth of prediction (cm)	Grid spacing/ resolution	Number of zsamples	R^2 prediction	Validation	Covariates	Fitting methods	References
Edgeroi	1500	100	25	341	0.26	Internal	Terrain attributes, Landsat images	Artificial neural networks (ANN)	(Minasny et al., 2006)
Edgeroi	1500	100	90	341	0.44	Internal	Terrain attributes, gamma radio-metrics, Landsat images	Artificial neural networks (ANN) & regression kriging	(Malone et al., 2009)
Peanut basin, Senegal	1030	20	30	155	0.12	External	Geomorphological units, slope position, vegetation,	Expert classification tree	(Mora-Vallejo et al., 2008)
Catchment, Inner Mongolia	3600	100	90	120	0.74	Internal	Land use, geology, soil groups, topography	Random forests	(Wiesmeier et al., 2011)
Santa Fe River Water-shed, Florida	3585	30	30	141		No	Landsat image, elevation	Regression kriging	(Vasques et al., 2010a)

Arctic	800	100	15	55	0.56	No	NDVI (ASTER)	Linear model	(Burnham and Sletten, 2010)
Bago-Maragle State Forests, South-eastern Australia	500	100	25	165	0.54	No	Geology, DEM, climate	Linear model	(McKenzie and Ryan, 1999)
Croplands, Luxembourg	420	5	2.6	325	0.89	Internal	Hyperspectral image	Partial least squares (PLS)	(Stevens et al., 2010)
Teramo province, Italy	100	50	40	250	0.7	No	Terrain attributes, Landsat	Regression kriging	(Marchetti et al., 2010)
Drenthe province, the Netherlands	125	90	25	2111	0.46	Independent stratified random sampling	Terrain attributes, groundwater class, land cover, soil type, paleogeography, geomorphology	Linear model	(Kempen et al., 2011)

Continued

Table 1.1 A review of recent studies on digital mapping of soil carbon—cont'd

Study area	Extent (km^2)	Maximum depth of prediction (cm)	Grid spacing/ resolution	Number of samples	R^2 prediction	Validation	Covariates	Fitting methods	References
Dry Creek Experimental Watershed (DCEW), Idaho USA	28	30	30	133	0.62	No	NDVI, potential insolation	Linear model	(Kunkel et al., 2011)
Sedgwick Natural Reserve, Santa Barbara, USA	20.6	A & B Horizons	2	20	0.78	No	Compound topographic index	Linear model	(Gessler et al., 2000)
Eucalyptus plantation, central Madagascar	15.9	30	30	41	0.61	Crossvalidation	Elevation, slope	Boosted regression tree	(Razakamanarivo et al., 2011)
Barro Colorado Island. Panama Canal	15	50	5	165		Crossvalidation	Topographic attributes, soil units, parent material, forest history	Random forests	(Grimm et al., 2008)

Eastern Kentucky	15	30	30	101	0.70	Internal	Landscape position, terrain attributes	Linear model	(Thompson and Kolka, 2005)
IA Watson, Narrabri	4.6	100	5	60	0.80	No	Eca, gamma radiometrics, terrain attributes	Decision tree	(Miklos et al., 2010)
Narrabri	2	10	30	146	0.73	No	Hyperion, Vis–NIR	PLS	(Gomez et al., 2008)
Crisp County, Georgia	1.15	15	2	28	0.98	External	Aerial photograph	Linear model	(Chen et al., 2000)
Wulfen, East Germany	0.7	Surface	6	72	0.9	Internal	Hyperspectral image	Linear model	(Selige et al., 2006)
Kalamazoo County, Michigan, USA	0.5	10	15	78	0.70	No	NIR	Principal component regression	(Huang et al., 2007)
Field 1, Nebraska	0.48	30	4	206	0.46	No	Relative elevation, ECa, and surface reflectance (IKONOS), and soil series	Regression kriging	(Simbahan et al., 2006)
Field 2, Nebraska	0.52	30	4	202	0.66	No	Relative elevation, ECa, and surface reflectance (IKONOS), and soil series	Regression kriging	(Simbahan et al., 2006)

Continued

Table 1.1 A review of recent studies on digital mapping of soil carbon—cont'd

Study area	Extent (km^2)	Maximum depth of prediction (cm)	Grid spacing/ resolution	Number of samples	R^2 prediction	Validation	Covariates	Fitting methods	References
Field 3, Nebraska	0.65	30	4	265	0.75	No	Relative elevation, ECa, and surface reflectance (IKONOS), and soil series	Regression kriging	(Simbahan et al., 2006)
Shiawassee River watershed, Michigan	0.12	20	4	134	0.52	Internal	Terrain attributes	Linear model	(Mueller and Pierce, 2001)
South-eastern Michigan	0.12	10	1	50	0.84	Internal	On-the-go NIR sensor, topography, aerial photograph	Linear model	(Muñoz and Kravchenko, 2011)
Belgian Lorraine region	0.06	5	2.6	68	0.75	Internal	Remotely sensed: Vis, NIR, SWIR (Short Wave Infrared)	PLS	(Bartholomeus et al., 2011)

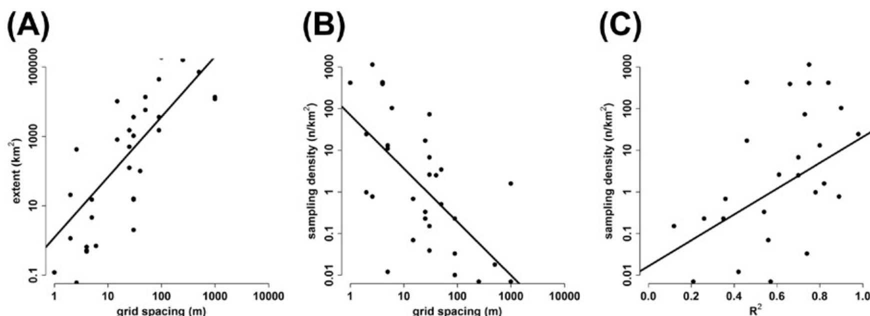


Figure 1.1 Results from previous studies on digital mapping of soil carbon: (a) the relationship between grid spacing (resolution) and extent of the studied areas, (b) the relationship between sample density and resolution of the digital soil maps, (c) the relationship between sample density and the goodness of fit (R^2) for the prediction of soil carbon. For color version of this figure, the reader is referred to the online version of this book.

2.2.4. Validation

Half of the studies do not show any validation, and the other half mostly used crossvalidation and internal validation (random holdback or data splitting).

2.2.5. Uncertainty

Most of the studies do not show any uncertainty of prediction. Only studies based on geostatistical mapping have uncertainty estimates, and most data-mining studies do not show any maps of uncertainty.

2.2.6. Covariates

Topography as manifested through various terrain attributes are generally the most widely used covariates. Land use or land cover and satellite images, (Normalized Difference Vegetation Index (NDVI) derived from remotely sensed images) also play an important role. Gamma radiometrics was also shown to be very useful. For field-scale fine-resolution mapping, remotely and proximally sensed visible to near infrared (NIR) reflectance has been shown to provide good estimates (Muñoz and Kravchenko, 2011).

In the proceeding sections, we will discuss in detail each of these factors and their influence on soil carbon mapping.



3. SOIL CARBON MEASUREMENT AND DEPTH

3.1. Soil Carbon Concentration Versus Density

Total soil carbon is usually separated into SOC and inorganic ($CaCO_3$) carbon. Soil carbon concentration or content can be expressed on a mass basis

by C_m (kg kg^{-1} or percent mass g 100 g^{-1}) or a volume basis by C_v (kg m^{-3}). The relationship between the two is derived from soil bulk density ϱ:

$$C\left(\text{kg C per m}^3\text{soil}\right) = C_m\left(\text{kg kg}^{-1}\right) \times \rho\left(\text{kg m}^{-3}\right). \quad (2)$$

We are usually interested in soil carbon density (C_d) as a measure of the amount of carbon stored; this is expressed as the integral of C_v to a depth z (in meters):

$$C_d = \int_0^Z C_v(z)\, dz, \quad (3)$$

where C_d in kg m^{-2} is the amount of carbon stored per unit land area. Laboratory measurement of total carbon in the soil is usually made by dry combustion, whereas SOC can be made by the wet oxidation method. Recently, visible, near- and midinfrared reflectance spectroscopy has been offered as an alternative, cheaper way to measure soil carbon (Bellon-Maurel and McBratney, 2011; Madari et al., 2006; Morgan et al., 2009; Reeves, 2010; Stevens et al., 2010). The infrared spectroscopy method is based on empirical calibration, where the spectra have been shown to correlate well with total, organic, and inorganic soil carbon contents (Morgan et al., 2009; Vasques et al., 2008). However, the first requirement is the need to establish a database of soil samples where their carbon concentration has been measured using the standard method. The infrared spectra of the soil samples in the library are then related to the standard carbon concentration using empirical functions. The calibration functions can then be used to predict soil carbon concentration for new samples, where only infrared spectra measurement is required (Bellon-Maurel and McBratney, 2011).

Most studies have mapped SOC or total C concentration or density. Because C concentration usually has a positive skewed distribution, most studies used a logarithmic transformation, although square-root transformation sometimes is more appropriate. Some studies have also mapped inorganic C concentration (Miklos et al., 2010; Rawlins et al., 2011) and C fractions, such as recalcitrant C, hydrolyzable C, hot-water-soluble C, and mineralizable C (Vasques et al., 2010b). Other C components maps also have been produced, for example, Carré et al. (2010) mapped the C/N ratio for forest litters in Europe and Angers et al. (2011) mapped the carbon saturation deficit of French agricultural top soils.

3.2. Soil Carbon Variation with Depth

Most studies on soil carbon mapping (Table 1.1) focused on the surface (top 10–30 cm), where soil carbon mostly accumulates. However, the

distribution of carbon at depths (>30 cm) also has an important role. Angers and Eriksen-Hamel (2008) reviewed studies that compared SOC distribution under no-till and full-inversion tillage; they showed that the SOC content was significantly greater under no-till than under inversion in the surface layers. However, at tillage depth and below, the average carbon content can be higher under full tillage than under no till. Meersmans et al. (2009) also pointed out that SOC in the subsoil seems to be strongly related to sorption capacity of pesticides and to denitrification capacity of leached components. Therefore, the knowledge of spatial distribution of soil carbon with depth is of great importance for carbon stock accounting and as inputs to hydrological modeling.

Some studies that examined soil carbon distribution at multiple depths, usually obtained their data from purposive-designed surveys with consistent depth sampling (Grimm et al., 2008; Vasques et al., 2010a). However, soil samples were usually collected based on horizons or fixed depth layers. Studies investigating relationships within legacy soil databases often drew together differing profile sampling approaches, such as sampling by genetic horizons or by varying depth increments, which may also contain samples noncontiguous with depth. Therefore, a soil carbon profile reconstruction method is required to harmonize such data.

Soil carbon has been observed to decline rapidly with depth; the concentration of carbon with depth is usually expressed as an exponential decay function. In one of the early studies, Russell and Moore (1968) found that the organic matter content from 63 profiles from Australia could be expressed as follows:

$$C = C_0 \exp(-kz), \tag{4}$$

where C_0 is the C concentration at the soil surface and k is the rate of decrease, z is depth. They reasoned out that this function is chosen because of its mathematical simplicity and its apparent similarity to the profile depth changes found for biological and related properties.

There are also other equations proposed to describe the decrease of soil carbon with depth, but they are just a variance of the exponential model (Arrouays and Pelissier, 1994; Bernoux et al., 1998; Zinn et al., 2005). Minasny et al. (2006) used a generalized negative exponential depth function:

$$C = C_a \exp(-kz) + C_b, \tag{5}$$

with conditions C_a, C_b, $k \geq 0$, where C is soil C content in volume basis (kg m^{-3}); z is the absolute value of depth from the soil surface (m);

$(C_a + C_b)$ kg m^{-3} is the C content at the soil surface; C_b is the C content at the bottom of the profile; and k (m^{-1}) is the rate of C decrease with depth.

A disadvantage of using the exponential depth function is that any local variation in the soil profile affects the quality of fit everywhere else in the profile (Webster, 1978). Consequently, they lack flexibility in fitting depth functions, and the quality of fit may be quite varied. Webster (1978) demonstrated that spline interpolators are better for some organic matter profiles of British soils, especially for the Podzols, where the exponential decrease assumption is invalid. Another matter that is usually overlooked is that usually the SOC data are derived from bulked samples taken from particular horizons or layers. It is assumed that the recorded C concentration represents the average value for the depth interval from which the sample was taken. When presented as a soil depth, horizon SOC data should be stepped, whereas soil in general varies continuously with depth. Ponce-Hernandez et al. (1986) proposed a nonparametric depth function, involving a variation of the spline function, called an equal-area spline to model soil attribute depth functions. This approach not only fits the soil C data with depth but it also disaggregates data obtained from horizon bulk samples into a continuous depth distribution. The key characteristics of the equal-area spline are as follows: it consists of a series of local quadratic polynomials with the 'knots' or 'positions of joins' located at the horizon boundaries, and the area of the fitted spline curve is equal to the area of the corresponding layer value, thus ensuring that the mean value of the horizon is maintained. Bishop et al. (1999) tested the ability of equal-area spline to predict soil depth functions based on bulk horizon data of three soil profiles. Their results indicated the superiority of equal-area splines in the prediction of depth functions. Figure 1. 2 shows an example of the equal-area spline fitted to observations of soil carbon from a legacy soil survey data in the Edgeroi area, Australia (Malone et al., 2009). The original samples were collected at various depth intervals; thus, the spline interpolation allowed the harmonization of carbon content at regular depths, which facilitated the prediction of soil carbon content at standard depths.

Breidt et al. (2007) developed a statistical procedure to account for carbon concentration on soil samples collected from varying horizons. They proposed a linear mixed model to estimate the total carbon concentration difference between two tillage systems at the depth of interval of 0–30 cm. The model used parametric fixed effects to represent covariate effects (depth, time, climate), random effects to capture depth correlation, and an

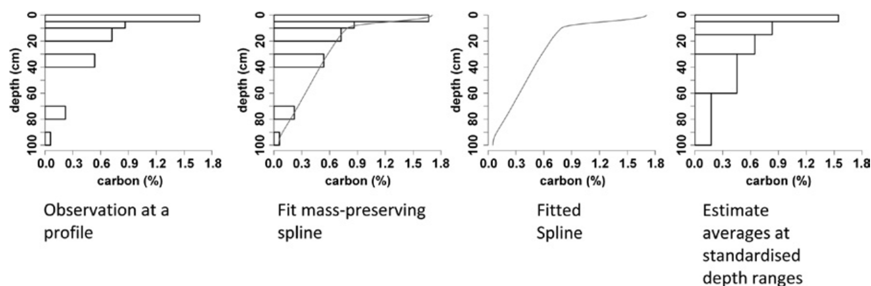


Figure 1.2 An example of equal-area spline fit to soil data and prediction of the soil C content at specified depth intervals. For color version of this figure, the reader is referred to the online version of this book.

integrated smooth function to describe effects of depth. The depth function is specified as penalized splines. The methodology is applied to the problem of estimating a change in carbon stock due to a change in tillage practice from traditional to no-till in the US.

3.3. Another Issue with Depth: The Mass Coordinate System

The calculation of carbon stock (carbon mass over an area) requires the information of soil bulk density. When we use standard depths for comparisons between sites and/or different times, variation in carbon density results can occur due to tillage, compaction, swelling/shrinking, and erosion. This is because the soil mass over certain depths will differ when there is a change in the bulk density, and therefore, comparisons of soil carbon masses in differing soil masses are not appropriate. For example, for two soils sampled to a depth of 10 cm with the same carbon content of 1 g 100 g^{-1}, but with bulk densities of 1.0 and 1.3 Mg m^{-2}, will return soil C masses of 10 and 13 kg m^{-2}. This difference is due to fluctuating soil masses within sampled depths.

The most popular approach in the soil carbon accounting literature is the equivalent soil mass (ESM) approach (Ellert and Bettany, 1995), which attempts to correct for differences in bulk density by calculating the mass of soil carbon in an ESM per unit area. This is done by first designating the mass of the heaviest soil layer as the equivalent mass. The carbon density from subsequent sampling is then calculated by estimating the thickness of the deepest soil layer required to attain the equivalent mass. The ESM method is quite cumbersome in recognizing the heaviest horizon, and when the boundaries of horizons are not distinct, this is not so simple. Additionally, the depth of the transition between horizons can change

over short distances. This can result in misinterpretation and miscalculation (Lee et al., 2009).

Gifford and Roderick (2003) proposed the use of the mass coordinate system, which is simpler and better for handling this issue. The material coordinate or Lagrange system was proposed in soil science literature by Smiles and Rosenthal (1968) for calculating the water flux in swelling soils. The approach is relatively well known in soil physics and has been applied in the calculation of water flow in swelling soils (McGarry and Malafant, 1987). For carbon accounting, the carbon density estimation can be based on the mass of the soil mineral materials. This is done in the following manner: first, the mineral mass of each sampling layer is calculated from the bulk density ρ_b (in kg m^{-3}), mineral fraction f_{min} (kg kg^{-1}), and thickness z (m) of the layer:

$$m = z\rho_b f_{min}. \tag{6}$$

The mineral fraction can be estimated from the fraction of the soil that is not organic matter. Next, the cumulative mineral mass for each layer M (in kg m^{-2}) can be calculated from

$$M_i = \sum_{l=1}^{i} m_l. \tag{7}$$

Similarly, the cumulative C density for each layer is also calculated. Afterward, the cumulative carbon density is plotted against the cumulative mineral mass (Fig. 1.3). The amount of carbon for a fixed mineral mass (e.g. 400 kg m^{-2} or 1200 kg m^{-2}) can then be easily calculated. Because we only consider the mineral mass, we exclude carbonates in the calculation. For organic soils, the amount of carbon should be large. In stony soils, we also do not consider materials >2 mm as the mineral mass. Figure 1.3 shows an example of the observations of carbon density that were translated to cumulative mineral mass and cumulative carbon density where the total carbon density to a fixed mineral mass can be readily calculated.

The mass coordinate method is a formal method and has been used for correcting water content changes in swelling soils (McGarry and Malafant, 1987) and for quantifying carbon losses (Smiles, 2009). The assumption is of course that the changes of density are isotropic, the carbon 'moves' together with the mineral material, and that there is no loss or gain of material at the soil surface. The cumulative mass approach should be preferred as the basis for carbon stock accounting and C density reported on a fixed mineral mass per unit area (e.g. see 2006 IPCC Guidelines; Egglestone et al., 2006).

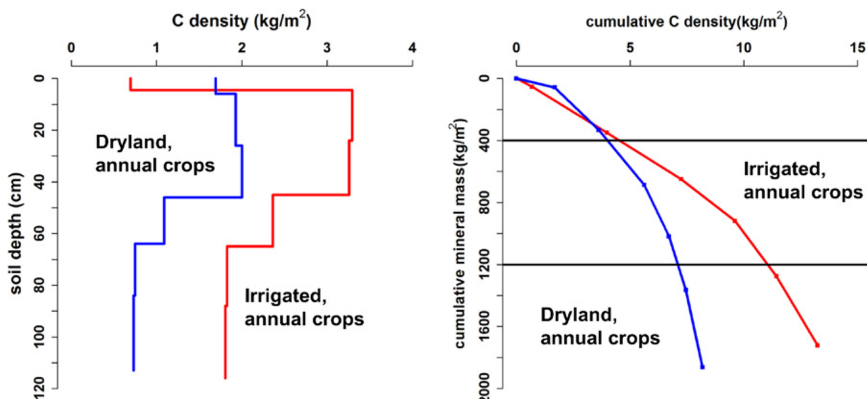


Figure 1.3 An example of the material coordinate system applied to soil carbon observations at 2 sites. Soil carbon densities collected at 6 depth ranges were converted to cumulative mineral mass and cumulative C density. Cumulative C density at specified mineral mass (e.g. 400 and 1200 kg m^{-2}) can be readily calculated. For color version of this figure, the reader is referred to the online version of this book.



4. SOURCE OF DATA: SOIL SAMPLING AND LEGACY DATA

4.1. Sampling in the Presence of Covariates

Here, we only provide a brief review on sampling approaches; a more comprehensive treatise on sampling can be found in De Gruijter et al. (2006). Sampling for carbon mapping can now be done more efficiently with the help of environmental covariates. In the absence of any information, grid sampling or geographical coverage is usually recommended (Walvoort et al., 2010). In the presence of covariates, stratification offers an effective way to cover the variation of soil carbon. The stratification divides an area into strata that are similar in covariate space. Each stratum is then sampled independently, out of which individual sampling units can be selected randomly. Stratified sampling can lead to more efficient statistical estimates (De Gruijter et al., 2006). One way to stratify the area of interest is by using numerical methods or cluster analysis to group the covariates into classes that are similar (Miklos et al., 2010; Simbahan et al., 2006). A fixed number of samples are then taken from each of the classes.

Minasny and McBratney (2006) proposed the use of a conditioned Latin hypercube sampling (cLHS) design to cover the covariate space. They argued that for the purpose of spatial prediction model calibration, it would be beneficial to select samples that cover the whole distribution of values of

each of the covariates. Latin hypercube sampling is a procedure that ensures a full coverage of the range of each variable by maximally stratifying the marginal distribution. The cLHS algorithm attempts to select *n* observations (sites) from the covariates that can form a Latin hypercube in the feature space. Samples obtained using the cLHS method were found to closely represent the original distribution of the environmental covariates (Brungard and Boettinger, 2010).

4.2. Legacy Soil Data

In many instances (due to constraints in budget and time), legacy soil data are the only source of data available to be used as an estimate of carbon stock baseline at a regional or continental scale. Using legacy soil data can be problematic as the data arise from traditional soil survey. There are no statistical criteria in traditional soil sampling, and this may lead to biases in the areas being sampled. Powers et al. (2011) conducted a meta-analysis of studies that quantified changes in soil carbon stocks with land use in the tropics and found that there is a strong geographical bias of the field observations that were highly unrepresentative of most tropical landscapes. The authors also strongly caution against generalizing average values of land-cover change effects on soil carbon stocks. This study highlighted the problem in using legacy soil data and recommends more representative sampling and monitoring schemes. Bui et al. (2009) meanwhile suggested that the SOC map generated using data-mining techniques based on legacy data, which were collected from traditional survey at different times, still represent a credible map even with a relatively sparse training data. The map produced could be considered as a baseline of SOC content.

Nevertheless, we can assess the reliability and quality of the legacy soil data based on the available covariates. Carré et al. (2007b) used the principle of hypercube sampling to assess the quality of legacy data; they derived a weighing factor of the legacy data based on their coverage in covariate space. First, the covariate space was divided into hypercubes based on the quantiles of the covariate. The occupancy of the legacy data in the hypercube was then checked to determine whether the legacy data occupied the hypercube uniformly or if there was overobservation or underobservation in the partitions of the hypercube. The Carré et al. (2007b) approach also allows the posterior estimation of the apparent probability of sample units being surveyed. This approach also allows the determination of where new sampling units should be located if there is a possibility of sampling investment.

5. PREDICTION AND MAPPING

5.1. Soil Carbon Variation

Soil carbon has a high spatial variation, and a review by McBratney and Pringle (1999) found that the spatial correlation of soil carbon within a field is between 20 and 300 m. This short-range variation is important for field-scale mapping and requires an efficient sampling to capture this variation. The spatial variation of soil carbon also changes with increasing extent, at a continental scale, and the spatial variation can be much larger. Figure 1.4 shows variograms of topsoil organic carbon content in Australia based on a nationwide legacy soil data (McKenzie et al., 2005). The variogram shows a high variation; with increasing variance up to 200 km. Variation in the North–South direction is much higher than the variation in the East–West direction. Overall, there seems to be no apparent "sill" as observed in a field (Fig. 1.4).

At a global scale, the variation of soil carbon can be larger than any regional scale observation; the carbon content fluctuates with latitude and

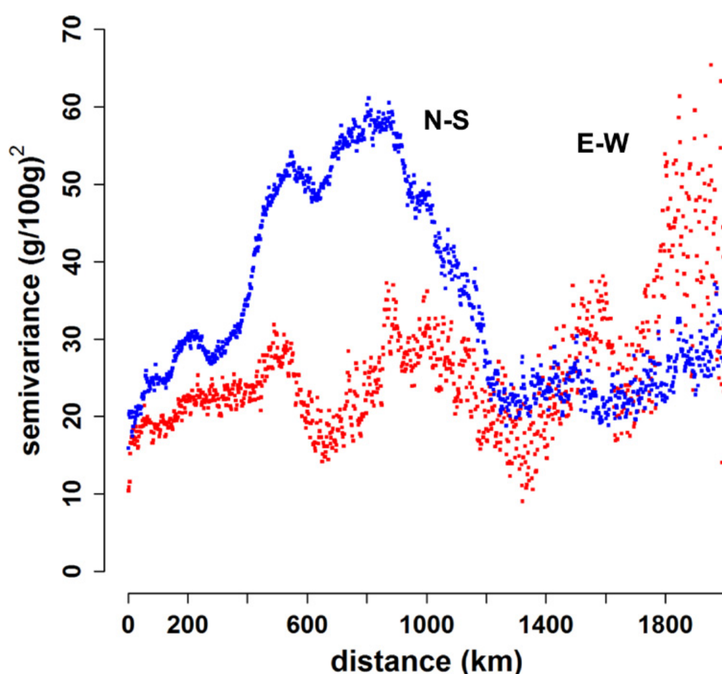


Figure 1.4 Variogram of topsoil organic C in Australia. For color version of this figure, the reader is referred to the online version of this book.

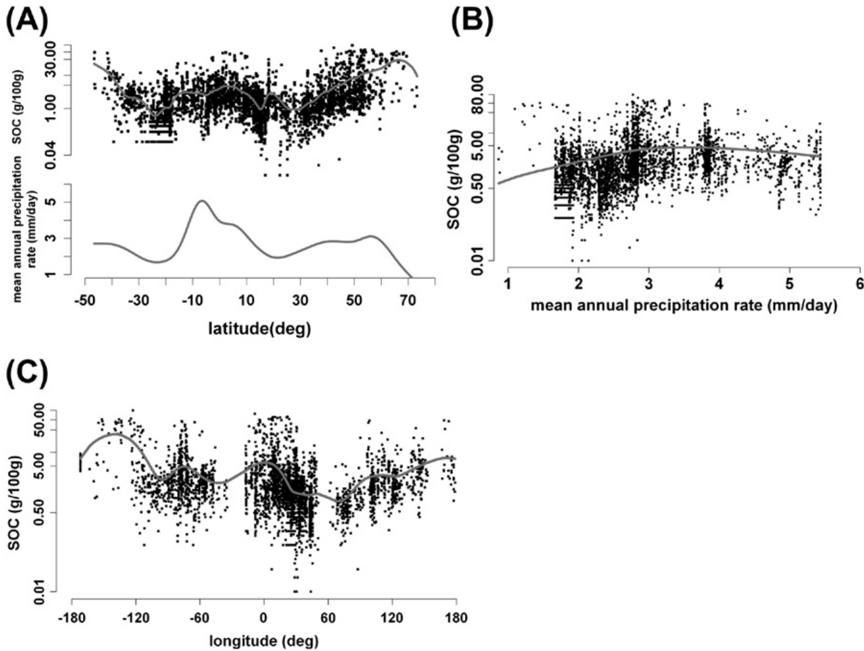


Figure 1.5 (a) The SOC content variation and mean precipitation rate along the latitude. (b) The relationship between mean precipitation rate and SOC. (c) SOC content along the longitude. SOC data from ISRIC–WISE global soil profile dataset (Batjes, 2008a), and the zonally averaged MAP rate is from Adler et al. (2003). For color version of this figure, the reader is referred to the online version of this book.

longitude (Fig. 1.5a, data from ISRIC-WISE global soil profile dataset, Batjes, 2008a). Soil carbon content is greater at higher latitudes, decreases in the midlatitudes, and increases in the tropics. Except for the extreme latitudes, the pattern follows the global mean annual precipitation (MAP)(Adler et al., 2003), where the tropics have a maximum precipitation and a peak around 50 N. The high carbon content at high latitudes corresponds to the low temperature regimes. When we plot the MAP rate along the latitude with soil carbon, we can see that soil carbon tends to increase with the MAP until around 1000 mm, above which the values plateau (Fig. 1.5b), which is also observed by Guo et al. (2006) in the US Soil carbon across the longitude Fig. 1.5c shows the variation over continents, with higher values in the west, and low values around Eastern Africa, and the values increase going toward the east. Figure 1.6 shows the variogram of surface soil carbon content, where the variance can be twice as large as that at the continental scale. The variation is significant, cyclic, and as expected, variation in the north–south

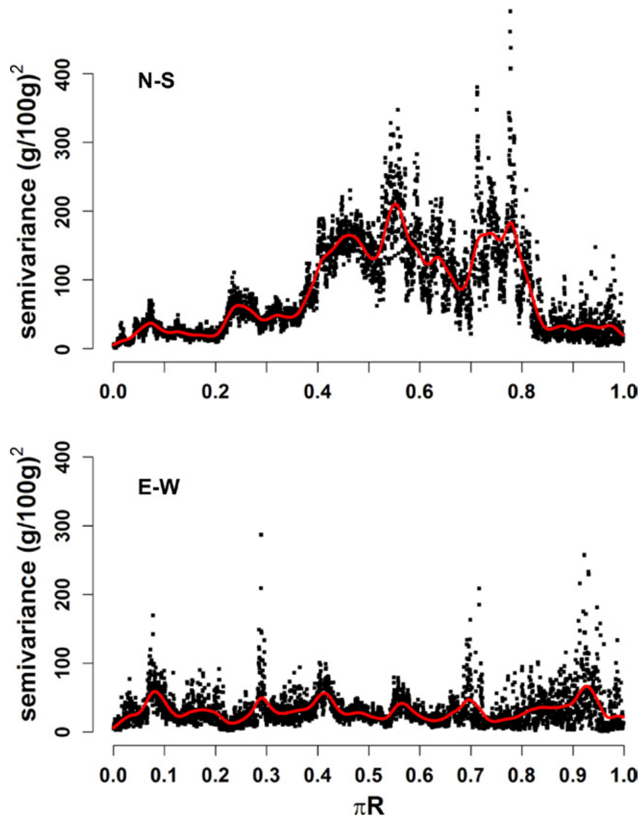


Figure 1.6 Global variogram of the surface soil C content for the north to south and east to west directions; R is the radius of the earth (6378 km). For color version of this figure, the reader is referred to the online version of this book.

direction is much greater than in the east–west direction. The variation in the north–south direction increases at several stages, increasing around 450, 1500, and 2500 km. Meanwhile, there is no clear pattern in the east–west variogram. The premise in digital soil mapping is that the environmental covariates should help in dealing with trends underlying this high variation.

5.2. Environmental Covariates

Jenny et al. (1968) presented one of the first empirical model for carbon which integrated factors of soil formation:

$C = k_0 + k_1$ MAP $+ k_2$ MAT $+ k_3$ parent rock $+ k_4$ slope $+ k_5$ Flora $+ k_6$ Latitude

where k are the empirical coefficients.

The model was calibrated against a moisture transect of 97 surface (0 – 20 cm) soil observations collected across the West Coast of USA ranging from desert to humid region. Jenny found that MAP was the most influential predictor followed by MAT, parent rock, and plant species. As shown in Table 1.1, most studies used elevation and its derivatives to predict soil carbon, and land use/land cover, also important covariates. The covariates are not only useful as empirical predictors, but they should have significant biophysical reasons as drivers of soil C distribution. The covariates can reflect the supply of organic matter to the soil (e.g. net primary productivity) and its potential decomposition (the effect of temperature and moisture). The role of climate becomes important when we look at the global to regional distribution of SOC (see also Fig. 1.5). Global and continental meta-analysis of studies in soil carbon levels after land-use change shows the importance of two critical climate variables: temperature and rainfall (Guo and Gifford, 2002; Powers et al., 2011). Continental scale prediction of soil carbon also revealed the importance of temperature and soil moisture indices as strong predictors (Bui et al., 2009). Bui et al. (2009) found that for topsoil carbon prediction in Australia, climate variables, such as annual mean moisture index, play an important role in delineating SOC zones. This relationship is hypothesized to be associated with plant primary productivity. Other important variables include elevation and lithology.

The study of Bui et al. (2009) also found that the spatial topsoil carbon pattern corresponds well with vegetation, suggesting that the biota drives and regulates the global biogeochemical cycles of elements. They proposed that because the SOC signature still exists from the original native vegetation, and the SOC pattern at the continental scale does not respond quickly to land-use change. This idea is supported by the study of Schulp and Veldkamp (2008), which explored the spatial variability of soil carbon in the Netherlands and found that historical land-use patterns explain a much larger part of the total SOC variability when compared with current land use. Meanwhile, Yang et al. (2007) found that climatic factors explained most of the SOC variation in the top 1 m; however, vegetation type was the stronger predictor when only considering the top 20 cm of the profile. Nevertheless, remotely sensed vegetation parameters (e.g. NDVI) are usually good indicators of primary and ecological productivity, and these data have been successfully used to predict SOC concentration (Bou Kheir et al., 2010; Burnham and Sletten, 2010; Kunkel et al., 2011).

In areas with large terrain variations, soil carbon is often well predicted by terrain attributes (Grimm et al., 2008; McKenzie and Ryan, 1999). Nyssen et al. (2008) working in the Ethiopian Rift Valley suggested that the most important

factor controlling carbon concentration over the studied area was related to the duration of land emergence, which was explained by elevation. The correlation between soil carbon and terrain attributes can also depend on the scale or resolution of interest. For small extent (resolution < 100 m), it is hypothesized that local terrain attributes (slope, aspect, curvatures) are good predictors of soil, where elevation is the driving force behind soil erosion processes. Aspect plays an important role in soil formation, as it creates microclimatic and vegetation differences. However, at resolutions > 100 m, the local terrain attributes become less important, and position in the landscape seems to be more important (the *n* factor of *scorpan*; Arrouays et al., 1995; Moran and Bui, 2002).

Powers et al. (2011) examined studies of soil C change due to land-use conversion in the tropics and noted that, in addition to precipitation, clay mineral composition (in part inherited from parent material) is another important variable that is statistically significant in delineating observations into groups. They classified observations into three groups of clay minerals: allophanic soils dominated by noncrystalline clay minerals that may stabilize soil C, highly weathered soils dominated by low-activity clay with low surface area and cation exchange capacity (CEC), and young to moderately weathered soils dominated by high-activity clay with high surface area and CEC. A very useful covariate that can indicate soil clay content and mineralogy is gamma radiometrics (Wilford, 2011; Wilford and Minty, 2006). Rawlins et al. (2009) found that radiometric K is the most important predictor of soil C in Northern Ireland, especially for organic rich soils. This was because of the good spatial correlation between gamma-ray attenuation and soil moisture, as water reduces the intensity of gamma-rays significantly more than air does. The SOC tends to accumulate in wet or waterlogged areas. Thus, gamma radiometrics is an important covariate for mapping organic soils. In another study in Finland, radiometric K was successfully used in delineating peat areas (Lilja and Nevalainen, 2006).

Remotely sensed visible to NIR reflectance has been shown to be able to map soil carbon over large areas (Bartholomeus et al., 2011; Gomez et al., 2008; Stevens et al., 2010). However, the challenge is of course to be able to remove the influence of vegetation cover from the spectra (Ouerghemmi et al., 2011). Groundwork has shown the feasibility of predicting soil properties using airborne hyperspectral data in areas with bare soil cover. Further, the prediction at areas with bare soil can be extrapolated to the whole field using geostatistical procedures (Lagacherie et al., 2012). At a field-scale, proximally sensed infrared spectra have also been used successfully to map soil carbon (Bartholomeus et al., 2011; Muñoz and Kravchenko, 2011).

In summary, the relationship between environmental covariates and soil carbon depends on the environmental conditions, resolution, and the extent of the study area see (Fig. 1.1). At the continental to regional scale, climate seems to be the most important driving factor: mainly rainfall and temperature. Soil clay mineralogy seems to be a significant driving factor. Gamma radiometrics have been found to be important predictors for carbon at various scales, because of the good correlation between gamma-ray attenuation with soil moisture and clay type. Native vegetation was suggested to present an important signature of carbon at regional scales. In the landscape scale, erosion and deposition also plays an important role. At farm scales, current land-use practices become an important controlling driver. Nevertheless, all these relationships can vary depending on the soil characteristics and their environment. With regards to the use of contemporary climate as a predictor Jenny (1980) wrote: "The computer's verdict of tangible linkages of soil properties to the state factors pertains to today's environment. Either the pedologically effective climate has been stable for a long time, or past climates are highly correlated with modern ones, or the chosen properties have readjusted themselves to today's precipitation."

5.3. Estimating Bulk Density

Carbon stock (the mass of carbon over a unit area) is the preferred currency in soil carbon mapping, as the total mass of carbon within an area can be calculated directly. Thus, bulk density is needed; however, most legacy soil data do not have a measurement of soil bulk density, which therefore needs to be predicted. Tranter et al. (2009) proposed the following model for the prediction of bulk density:

$$\rho_{\mathrm{b}} = \rho_{\mathrm{m}} + \Delta\rho + \varepsilon, \tag{8}$$

where ϱ_{b} is the soil bulk density, ϱ_{m} is the typical mineral soil bulk density $\approx f$ (particle size distribution, depth), $\Delta\varrho$ is the variation associated with structural component $\approx f$ (organic carbon, tillage, etc.), and ε is the residual variation. The predicted bulk density for ϱ_{m} can be considered to be a typical value for a soil with a given particle size distribution, depth, and average structural features. The typical equation for mineral bulk density is usually in the form of

$$\rho_{\mathrm{m}} = a + b\ \text{sand}\ + c\ \text{sand}^2 + d\log(\text{depth}), \tag{9}$$

where a, b, c, d are empirical parameters. This function is defined such that bulk density increases with increasing sand content in a quadratic manner and also increases exponentially with depth as a result of overburden pressure. The $\Delta\varrho$ component is introduced to account for bulk density variation as a function

of soil carbon change. A simple model was proposed by Stewart et al. (1970) and Adams (1973) relating mineral bulk density and organic matter content:

$$\rho_b = \frac{100}{\frac{OM\,\%}{\rho_{OM}} + \frac{(100 - OM\,\%)}{\rho_m}}, \tag{10}$$

where OM% is organic matter percentage, and ϱ_{OM} is organic matter bulk density = 0.224 g cm^{-3}. This model merely shows an increase in specific volume of 0.06 cm^3/g with a 1% increase in soil carbon concentration. These relationships (Eqns (9) and (10)) were found to fit well for data from Australia (Tranter et al., 2007), Europe (Hollis et al., 2012), and the tropics (Minasny and Hartemink, 2011). The advantage of using the above relationship is that the mineral bulk density can be defined for each soil type (Hollis et al., 2012), and the variation of soil carbon can be incorporated independently. Some publications, for example, Moreira et al. (2009) suggested that bulk density can be predicted from infrared spectroscopy. We would be cautious of such a relationship, although in principle spectral calibration is a type of pedotransfer function, we cannot infer the physical relationships as demonstrated above.

5.4. Mapping Soil Depth Function

Most soil carbon mapping is performed in 2-D, where carbon concentration or stock is mapped for a prescribed depth interval. However, it would be beneficial if we could map the carbon content as a continuous function of depth, as soil carbon stock can be readily calculated at any depth. In an unpublished presentation, Barson et al. (2004) compiled a database of soil profiles to estimate the size of the Australian soil carbon pool. A linearized version of a negative exponential depth model was fitted to the profile SOC data. The parameters of the exponential model were then predicted by using environmental variables. These predictive equations were combined with continental surfaces for bulk density, clay content, pH, elevation, and climatic parameters to predict SOC stored at different depth across the Australian continent at a grid spacing of 5 km.

Minasny et al. (2006) used the negative exponential depth function (Eqn (5)) to describe soil carbon concentration variation with depth in the Edgeroi area, Australia. They then mapped the parameters of the exponential function using a modified neural network approach, where the functions were calibrated to predict all the parameters simultaneously and to fit the soil C concentration. This approach takes care of the parameter correlation. They predicted parameters of the exponential function (Eqn (5)) over the

whole area, which enabled them to calculate the C distribution over the profile and also the storage of C at any depth. In effect, this creates a pseudo-3-D soil carbon map.

Following this work, Mishra et al. (2009) fitted an exponential function to soil profile data from Indiana, US, and then interpolated the parameters independently using ordinary kriging. A better solution is to interpolate the parameters simultaneously using cokriging (Webster and Oliver, 2007). The procedure in cokriging is slightly more complicated, and mapping them in large areas with sparse observations tends to oversmooth the reality. Meersmans et al. (2009), unaware of the previous works, developed empirical functions that predicted the parameters of the exponential depth function for the area of Flanders in Belgium. The functions were stratified based on land use, and the parameters were related to particle size distribution and height of groundwater.

Realizing the limitation of the exponential depth function, Malone et al. (2009) fitted an equal-area spline function to the soil profile data (Section 3.2). Parameters of the spline function were then interpolated or predicted for the whole area. Following this work, Kempen et al. (2011) developed a method for mapping depth functions based on pedological knowledge combined with geostatistical modeling. Their approach is useful in areas, such as the Netherlands, where soil properties do not vary smoothly with depth, because of anthropogenic or geologic disturbance. They modeled the distribution of soil organic matter content for each of the typical soil horizons. Five depth function building blocks were defined, and for each soil type, the depth function structure was obtained by stacking a subset of model horizons. The parameters of the depth function for each of the horizons were interpolated using a geostatistical procedure.

Although we can model the distribution of carbon with depth, generally, the prediction accuracy decreases with depth (Kempen et al., 2011; Minasny et al., 2006; Wheeler et al., in press). This indicates that the environmental covariates mainly explain the soil conditions in the top 30–50 cm. The environmental covariates seem to have lost their elucidation power in the lower parts of the profile (Vasques et al., 2010a). The challenge remains to find potent covariates that can explain subsurface soil variation.

5.5. Global Mapping of Soil Carbon

The most widely used global map of soil carbon is based on the 1:5 million map produced by the Food and Agricultural Organization (FAO) and United Nations Educational, Scientific and Cultural Organization

(UNESCO) in 1981. In the mid-1990s, a 9-km raster version was produced by the FAO. The digitized FAO–UNESCO map is still widely used for global studies on such topics as climate change, world food production, and environmental impact assessment (Batjes, 1996). There are also more recent global maps of the SOC, including SOC density up to a 1-m depth at a resolution of 1 km (Scharlemann et al. 2009). The IPCC (Intergovernmental Panel on Climate Change) released a global above- and below-ground biomass carbon density that was rasterized to a resolution of approximately 1 km (Ruesch and Gibbs, 2008).

GlobalSoilMap.net is a new project developed by a consortium, which aims to create a digital map of the world's soil properties. This global effort will provide access to the best available map of soil properties across the globe at a resolution (grid spacing) of 3 arc second (~90 m) along with its confidence of prediction in a consistent format at the depth ranges of: 0–5, 5–15, 15–30, 30–60, 60–100, and 100–200 cm. Soil carbon concentration is one of the key properties that will be mapped.

Realizing that it is a significant effort to apply the *scorpan* prediction function across the globe, the approach taken (for global soil mapping) is a pragmatic one (Minasny and McBratney, 2010). The methods that are used (or will be used) for mapping consider the nature, availability, and density of existing legacy soil data. Figure 1.7 shows a first approach of mapping soil carbon in the USA based on a 1:250,000 soil map from the USDA–NRCS (United States Department of Agriculture - Natural Resources Conservation Service), where the soil polygons were converted to raster (resolution 90 m) estimates of organic carbon content (based on the STATSGO2 database) for the 6 depth intervals of the GlobalSoilMap.net specification. Currently, this effort is mirrored elsewhere in the globe.

5.6. A Regional Example

Here, we present an example of a pseudo-3-D soil carbon mapping in the Edgeroi area of Australia (Malone et al., 2009). This area of 1500 km^2 was covered by 341 soil profile observations, from which 210 are arranged on a systematic, equilateral triangular grid with an approximately 2.8-km spacing between sites, and 131 are distributed more irregularly or on transects. Soil samples were taken from the profiles at depths: 0–0.1, 0.1–0.2, 0.3–0.4, 0.7–0.8, 1.2–1.3, and 2.5–2.6 m, from which the soil physical and chemical analyses were conducted. SOC concentration and density were mapped at a grid spacing of 90 m on point support using various environmental covariates (digital elevation model and its derivatives, Landsat TM images, and

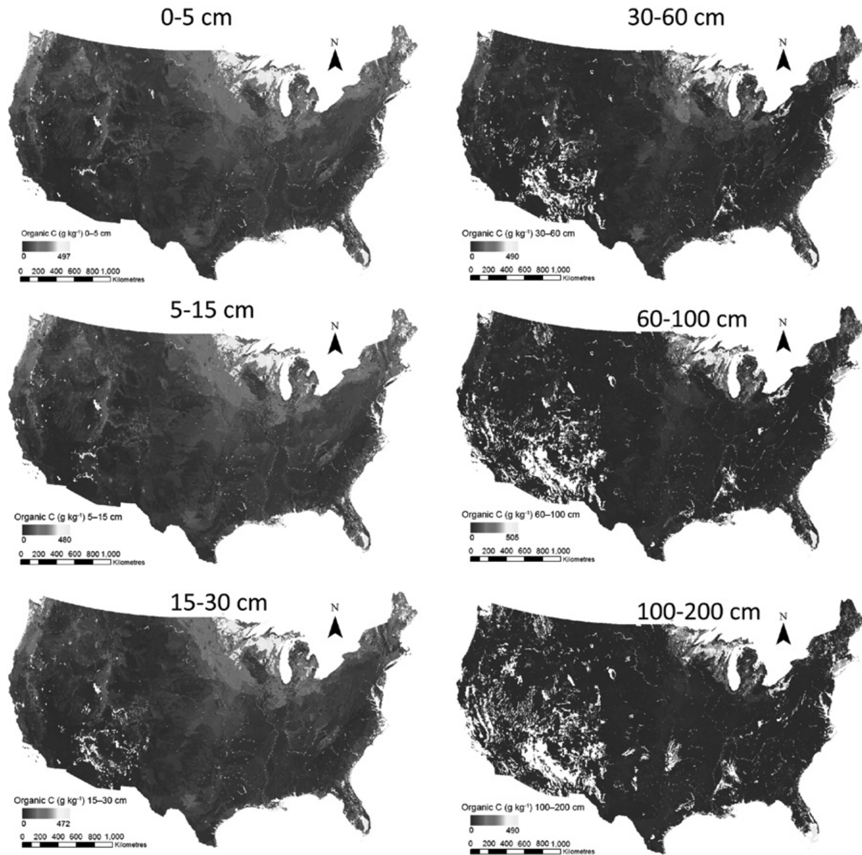


Figure 1.7 Map of soil carbon in North America at 6 depths based on GlobalSoilMap.net specifications. For color version of this figure, the reader is referred to the online version of this book. (*Figure courtesy of Nathan Odgers & Jon Hempel, USDA–NRCS.*)

gamma radiometrics) as predictors. The steps taken to produce the SOC maps were as follows:

- Compiling the soil observation data and relevant environmental covariates;
- Calculation of bulk density using pedotransfer functions (Tranter et al., 2007);
- Calculation of the SOC on volume basis (kg m^{-3}) using predicted bulk density;
- Fitting of the equal-area spline to the SOC and bulk density profile data;
- Joining the observation and covariates to form a calibration dataset;
- Deriving spatial prediction models from the calibration dataset to predict parameters of the depth function for the SOC and bulk density from environmental covariates;

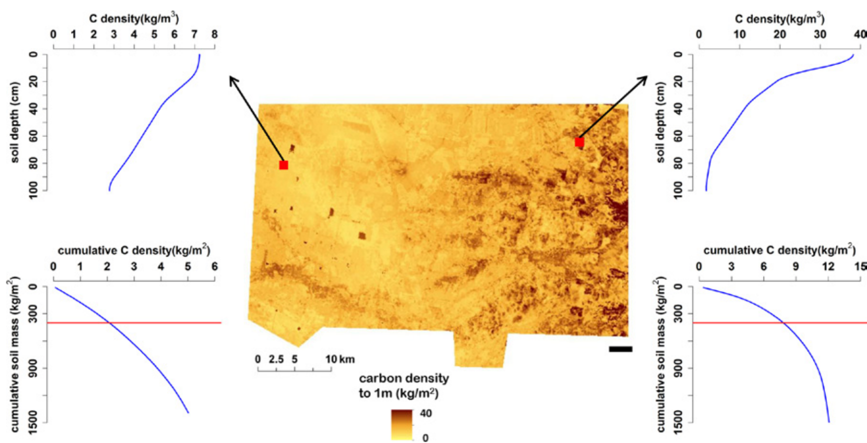


Figure 1.8 Map of soil carbon stock in Edgeroi, Australia. The semi 3-D approach allows the estimation of continuous depth of soil carbon density; based on a map of the bulk density, the total C density at any cumulative mineral mass can be readily calculated. For color version of this figure, the reader is referred to the online version of this book.

- Using the generated spatial prediction models to map the whole area for the SOC content and bulk density; and
- Calculating the uncertainty of the prediction (Malone et al., 2011).

The approach here mapped the SOC on volume basis and bulk density, as we can derive soil carbon density to any depth by integrating the SOC on volume basis. Using the mass coordinate approach, the cumulative soil mass and cumulative C density can be easily calculated see (Fig. 1.8). Figure 1.9 shows that by mapping the continuous function of carbon across the area, we can make nonlinear queries that cannot be done easily using conventional approaches, for example, the calculation of the depth at which cumulative mineral soil mass = 400 kg m^{-2} and the corresponding cumulative C density.



6. UNCERTAINTY AND VALIDATION

6.1. Uncertainty

An important output of digital soil mapping product is the availability of an uncertainty or measure of confidence in prediction. However, most of the studies (Table 1.1) do not show any uncertainty of prediction even though they are critical in determining the prediction confidence (Goidts et al., 2009). Unless the method is based on geostatistical studies, most data-mining studies do not show any estimates of uncertainty. Statistical methods for uncertainty analysis include but are not limited to Monte Carlo

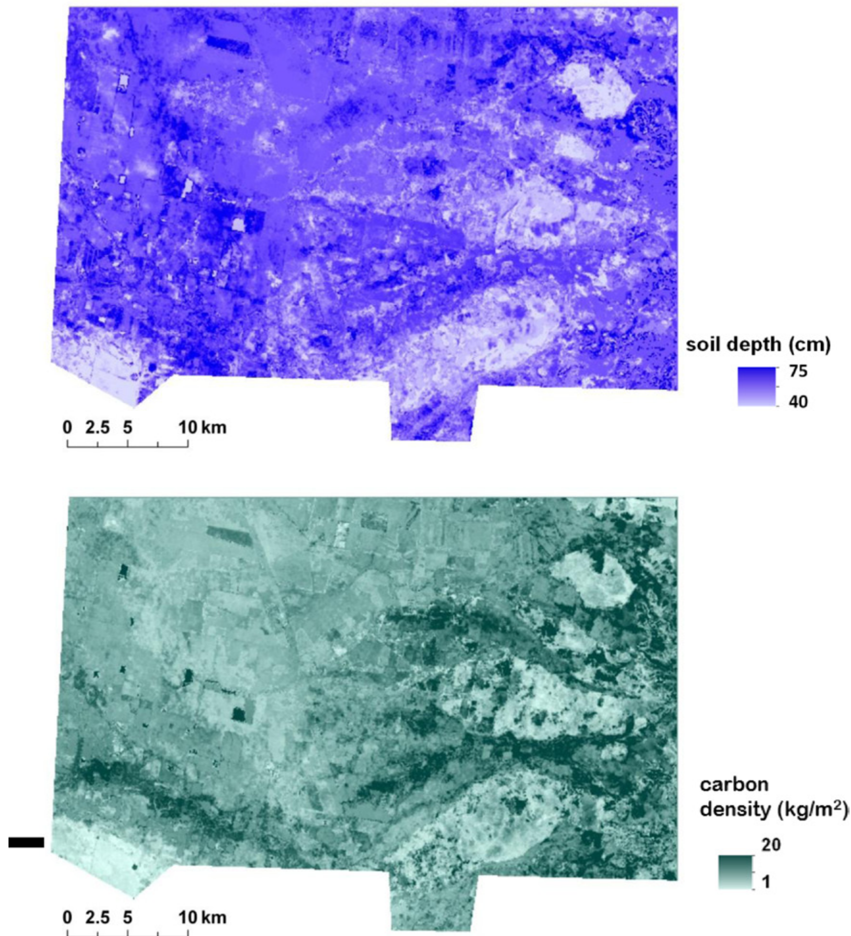


Figure 1.9 Mapping soil with a continuous depth function allows for nonlinear queries, such as (a) the depth at which cumulative mineral soil mass = 400 kg m^{-2} and (b) their corresponding cumulative C density. For color version of this figure, the reader is referred to the online version of this book.

simulation, bootstrapping, or Bayesian approach. Uncertainty analysis also allows the identification of the main source of error in the prediction. This is illustrated in the study of Goidts et al. (2009) who used an error propagation method to quantify the relative contribution of each of the variables and their interaction involved in estimating the SOC stock (SOC concentration, sampling depth, bulk density, and rock fragment content). They found that the main sources of uncertainty are the variability of the SOC concentration (due to errors from the laboratory and spatial variability) and of rock fragment content.

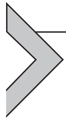
In a mapping study, Meersmans et al. (2009) calculated the parameter uncertainty of the exponential depth function using a Monte Carlo simulation. For mapping large areas, Monte Carlo simulation and the Bayesian approaches can be computationally too expensive as they require maps to be generated for each of the realizations (in the order of 1000–10,000 simulations). Malone et al. (2011) proposed the use of an empirical approach to derive the estimates of uncertainty. Uncertainty in this case is treated as the probability distribution of the output model errors, which comprises all types of uncertainty (model structure, model parameters, and data). This is particularly useful when we are dealing with data-mining tools in combination with the regression-kriging approach, where it is difficult and impossible to derive an analytical model for the parameter uncertainty. The idea behind this approach is to partition the model input (covariates) space into different clusters having similar values of model errors. The covariates used for prediction is partitioned into several classes using the fuzzy k-means with extragrades algorithm (McBratney and De Gruijter, 1992). Each class is then represented by a prediction interval determined from the empirical distribution. The fuzzy k-means with extragrades method is also used to identify and sufficiently penalize those observations outside the domain of the calibration data. Using the class centroids, a new observation can be allocated memberships to each of the established classes. Prediction limits for new observations then can be calculated as a weighted average of the membership values.

6.2. Validation

Half of the studies on soil carbon mapping (Table 1.1) do not show any validation. The other half of the studies used internal validation using random holdback. Validation of soil maps can be done in different ways:

- Crossvalidation, which can be leave-one-out or n-fold crossvalidation (Efron and Tibshirani, 1994). In leave-one-out crossvalidation, a sample point is left out, whereas the rest is used to calibrate the prediction model; the left-out sample is used to assess the accuracy of the calibrated model. The process is repeated for all samples. Meanwhile, in n-fold crossvalidation, the dataset is divided into n section or fold, and the crossvalidation process is repeated for n-folds.
- Internal validation using data splitting or random holdback, where a portion of the data (usually 30%) are randomly held back and excluded from model calibration. These holdback data are used to check the accuracy of the model.
- Independent sampling, where additional samples or observations are collected to check the accuracy of the model.

As explained by Brus et al. (2011), crossvalidation and random holdback may not provide unbiased estimates of map accuracy because of the nature of the data used. The prediction errors will generally be spatially correlated, and the data itself can be biased. Thus, they recommended the use of a probability sampling scheme to collect additional samples that can provide an unbiased quality measure of the map. Because of the high expense of resampling, only few studies (e.g. Brus et al. (2011) and Kempen et al. (2011)) have afforded independent sampling for the validation of soil C maps using a stratified simple random sampling approach.



7. MAPPING AND PREDICTING SOIL CARBON CHANGE

7.1. Mapping Soil Carbon Change

Mapping soil carbon change over an area can be done properly for areas with a monitoring scheme (Martin et al., 2011). Spatiotemporal models can only be applied in an area with a proper monitoring network. For example, Bellamy et al. (2005) used data from the National Soil Inventory of England and Wales obtained between 1978 and 2003 to show that carbon was lost from soils across England and Wales over the survey period at a mean rate of 0.6% yr^{-1}. Soil carbon change can also be mapped in countries having a comprehensive national scale database of soil carbon concentration sampled at different times (Meersmans et al., 2010). Fantappiè et al. (in press) used a database of Italian soils to map the SOC content between two periods (1979–1990 and 1991–2009) and showed that climate change generally had a small influence on SOC variations.

Legacy soil data collected at uneven space and time intervals can sometimes be used to indicate temporal changes. Lindert (2000) compiled a database of topsoil properties from Indonesia, which were collected from 1930 to 1990. Using regression models, he showed the decline in soil organic matter and N content with time in Java and the increase in total phosphorus and potassium. This work was criticized by soil scientists, as the soil test results came from various places at various times, which create problems such as possible systematic bias due to unrepresentative repeated sampling, change in sampling depth, and/or change in analytical method over time (Dobermann, 2002). Nevertheless, recent work in France has shown that a nationwide soil test database, which was collected for soil fertility assessment, can be used to detect decadal spatiotemporal changes in soil carbon (Saby et al., 2008). Minasny et al. (2010) used legacy soil data of the SOC in Java collected by the Indonesian Center for Agricultural Land Resources & Development

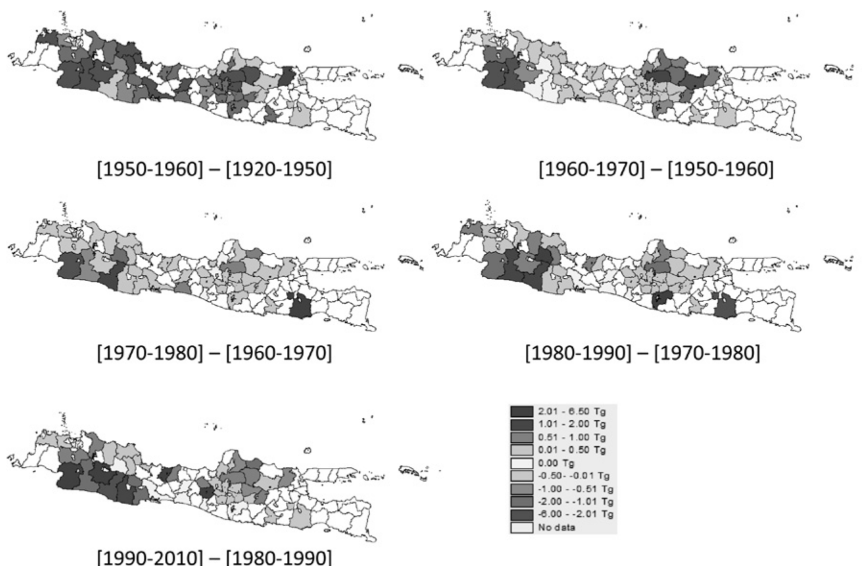


Figure 1.10 The changes in the C stock over successive periods for the top 10-cm soil in Java, Indonesia. Data based on Sulaeman et al. (2010). For color version of this figure, the reader is referred to the online version of this book.

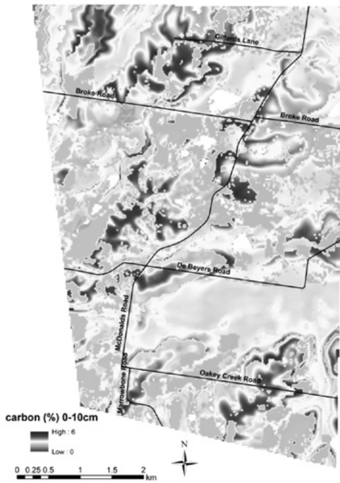
from 1923 to 2007. They aggregated the data into spatial administrative entities as most of the data do not have proper geographical coordinates, and mapped the changes in topsoil carbon content per decade (Sulaeman et al., 2010). Spatial analysis (Fig. 1.10) showed the trend of the SOC over the island with an apparent decline of the SOC concentration from around 2% in 1930–1940 to 0.7% in 1960–1970. However, there is an increase in the SOC content after 1970, with a median level of 1.1% in the 2000.

The variation of the soil C over time also has another implication that we can only use data from the relevant period for digital soil mapping. An alternative will be to standardize the C level collected at various times to a common period. A simple way is by adjusting the mean of the data at various periods to a desired period. A more elaborative option is to run a dynamic simulation model and predict the likely soil carbon at a common time.

7.2. Predicting Soil Carbon Change

Digital soil mapping only maps soil carbon status at a particular time; however, we can use the map to predict the likely carbon change. There are two ways we can approach this:

(1) Dynamic–mechanistic simulation model



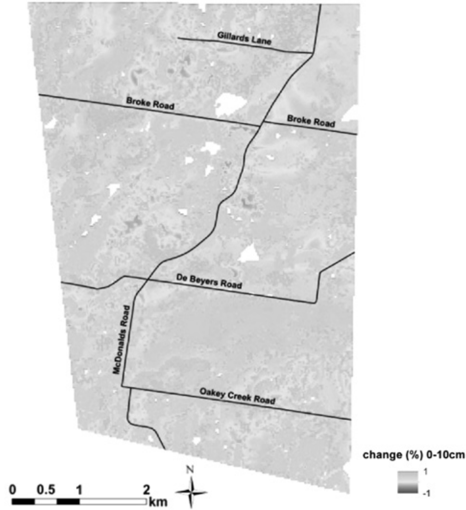


Figure 1.11 Predicted soil organic C content 0–10 cm for an area in the Hunter Valley, and simulated changes with land-use change. For color version of this figure, the reader is referred to the online version of this book.

The map of SOC can be fed into a dynamic–mechanistic simulation model. Most SOC simulation models do not consider a spatial aspect. Although there is work that incorporate SOC models into a GIS environment (e.g. Huber et al., 2002), the models still run as a one-dimensional component with no spatial connection (Tonitto et al., 2010). The SOC model is executed individually at each pixel based on the likely scenario, for example, increased temperature, or change in land management or land use (C input). This is the most widely used approach as indicated by a meta-study by Grunwald (2009). Landscape information can be incorporated by including a spatial process, for example, erosion and deposition. This is demonstrated by Walter et al. (2003) who performed field-scale simulations of the spatiotemporal evolution of topsoil organic C at the landscape scale over a few decades and under different management strategies. Figure 1.11 shows an SOC concentration map at 0–10 cm of an area in the Hunter Valley, Australia, which was generated using a digital soil mapping approach. We used this map for a scenario modeling, where the current vineyards are no longer profitable and may be changed to pasture. Using a simple 2-compartment model (Hénin and Dupuis, 1945), we estimated the SOC change for the next 10 years:

$$\mathrm{d}C/\mathrm{d}t = hI - kC, \tag{11}$$

where dC/dt represents the change in the SOC over time, and *I* is the annual C input, *h* is the isohumic coefficient, and *k* is the decomposition constant. The value of parameters *h* and *k* depend on the type of organic matter, soil type, temperature, and other environmental variables. Some values are suggested in the literature (e.g. Andriulo et al., 1999). The mean SOC level for this area in 2010 is 2.9 g 100 g^{-1} and the estimated SOC level in 2020 is 3.3 g 100 g^{-1}. Using this rudimentary approach, we can identify areas suitable for carbon sequestration and can estimate its carbon sequestration potential.

As most soil carbon dynamic models do not have a spatial component, and only simulate topsoil condition, Viaud et al. (2010) suggested a landscape-scale modeling approach to take into account the transfer and transformation processes in the SOC of a landscape. They proposed the design of a three-dimensional, spatially explicit representation of the landscape system with the integration of functional interactions and organic matter transfer functions into the conventional SOC modeling framework. Rudimentary landscape models of SOC dynamics as a component of long-term soil genesis models have been presented by Minasny et al. (2008) and Yoo et al. (2006). A spatial carbon model that includes production and input (*I*), decomposition (*k*), vertical mixing/bioturbation (q_m), and gains and losses (q_a and q_l) can be expressed as follows:

$$\mathrm{dC/dt} = I - kC + q_m + (q_a - q_l)\,. \tag{12}$$

The availability of such a model should help us make a better prediction of the soil C change in a landscape or region. Nevertheless, currently, there is still a disconnection between soil C mapping and mechanistic C dynamics modeling (Walter et al., 2006).

(2) Static-empirical model

The scorpan approach can also be used to infer the likely changes in soil properties over time as discussed in McBratney et al. (2003) termed 'partially dynamic soil scenario maps.' This advantage has not been explored yet; if we know any of the changes of the *scorpan* factor over time, we can project the existing soil map forward Δ*t*. For example, a change in the temperature due to climate change can be projected onto a *scorpan* model by calculating $c + (\Delta c/\Delta t)$ for all points in the map and running the prediction function. Similarly, for land-use change, we can infer the C change by calculating the change in the o factor: $o + (\Delta o/\Delta t)$. The C level at Δ*t* can be calculated as follows:

$$\mathrm{C}_{t+\Delta t} = \mathrm{C}_t + \Delta c/\Delta t + \Delta o/\Delta t\,.$$

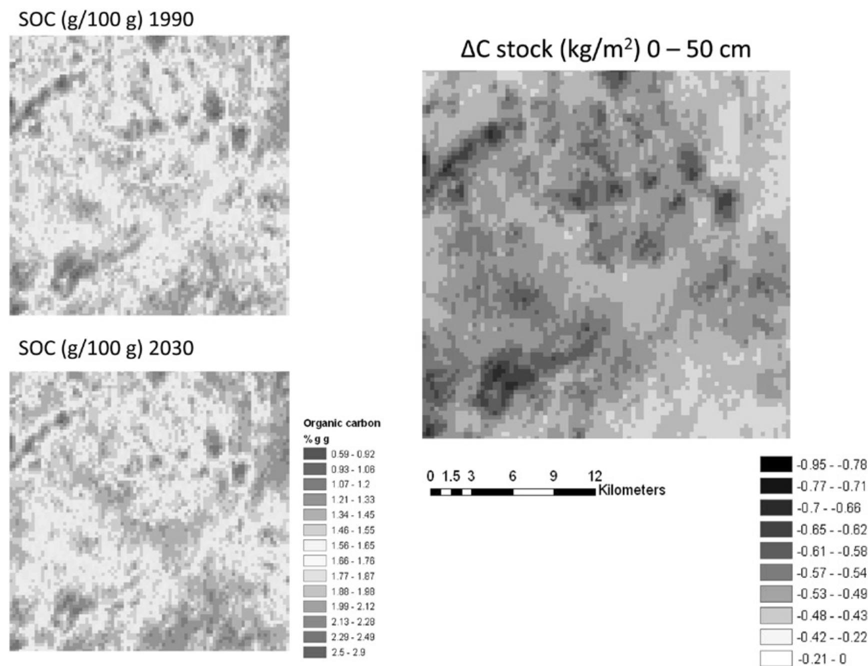


Figure 1.12 Map of the SOC at 0–50 cm in the southern part of NSW. The 1990 map was generated using a rule-based model based on legacy soil data and environmental covariates. The C level in 2030 was estimated under a likely climate change scenario (increase in the MAT and decrease in the MAP). The rule-based model was recalculated to reflect the climate change, and a map of likely C status in 2030 was produced. The change in carbon stock was then calculated based on estimates of the bulk density. For color version of this figure, the reader is referred to the online version of this book.

We demonstrate this approach by showing an example of projecting the influence of climate change on soil C level in a region of New South Wales (NSW), Australia. The baseline SOC concentration map (0–50 cm) was derived using legacy soil data collected in a nationwide database (McKenzie et al., 2003). A map was derived using a rule-based model based on the legacy data and environmental covariates with a grid spacing of 250 m (Wheeler et al., in press). Climate information was found to be important predictors in the spatial prediction model. See Wheeler et al. (in press) for the techniques involved in the mapping. We focused on an area of 49,000 ha in the southern part of NSW (Fig. 1.12) and estimated the likely change in soil carbon with climate change. We used the 50th percentile of the estimated likely change in the mean annual temperature (MAT) and MAP by 2030 (relative to 1990 levels) for the corresponding area of

NSW (Watterson et al., 2007). For a high emissions scenario, the MAT is expected to increase 0.6–1 °C (average 0.8 °C) and MAP to decrease 2–5% (average −3.5%). Using this information, we adjusted the climate covariates and recalculated the SOC concentration from the rule-based model. The assumption in this case is that the C level is at a steady-state condition (amount of input is constant). The projected map shows that there is an average potential loss of 5 t of C per hectare. Such information will help decision makers to decide on alternative land-management techniques that could reduce this loss.

This empirical approach is a relatively quick and pragmatic way to produce a first-cut scenario to predict the change in soil C. It has limitations compared with a dynamic simulation model, such as lack of feedback and possible extrapolation problems. Nevertheless, the *scorpan* approach has been locally calibrated, and any changes in the factors should reflect the changes in the predicted C.

Grunwald et al. (2011) recently proposed a space–time modeling framework (soil, topography, ecological and geographic properties–atmospheric, water, biotic, and human; STEP-AWBH), which attempts to incorporate anthropogenic factors to predict soil properties of the future. In their approach, the soil prediction function is estimated from various spatially explicit environmental variables, which can be grouped into 2 classes: STEP (parent material and geologic properties) factors that tend to be static within a human time frame and is thus represented in the model at one time, AWBH factors account for space and time, whereby the time component may be aggregated to represent different time vectors. Each of the AWBH factors can be represented by various covariates, such as climate, soil moisture, land use.



8. CONCLUSIONS

Digital soil mapping has taken off in the past decade, thanks to the advances in computing, spatial databases management, and numerical modeling. Digital mapping of soil carbon has quickly moved from a research stage to being operational with maps of carbon concentration and carbon stock being produced at various places in the world from field to regional and continental scale and extent. The *scorpan* spatial soil prediction function has been used to different extents and resolutions. Mapping has also evolved from a simple 2-D map to a pseudo-3-D representation.

From this review, we outline several important points that need to be considered to advance the art and science of mapping soil carbon:

- Obtaining representative soil samples for spatial modeling is important. The soil–landscape relationships built using the *scorpan* model only describes the empirical correlation found from the data. Biased samples will also make the model biased toward the observations.
- Digital maps produced using the *scorpan* approach mostly will reflect the covariates. If the covariates are poor, then the resulting map will also show numerous artifacts and their associated inadequacies.
- The various scales of covariates, the relationship between soil observation and covariates can change with the scale of the covariates. In some cases, covariates at a larger scale can be more useful than at a detailed fine resolution. There is still a need for more work to examine the spatial decomposition of covariates (Mendonça-Santos et al., 2006).
- The dynamics of soil carbon with time means that we need to consider the time dimension in our maps. Questions were raised on how to use and to standardize legacy data collected at different times? The integration of digital soil mapping and mechanistic soil–landscape modeling will help solve this problem.
- Mapping and estimating change: digital soil mapping should be able to provide a semidynamic model to estimate how soil carbon will change when one or several of the *scorpan* factors evolve with time.
- Models incorporating the mechanistic simulation model for spatial prediction can enhance our understanding of soil carbon distribution. As we have learned from empirical studies, the covariates used by data-mining models can infer the biophysical drivers of soil carbon distribution. However, we can also use our dynamic understanding of the soil–landscape system to better understand and predict the distribution and changes. We should be able to use a more mechanistic and dynamical relationship analyzing the spatial (and temporal) carbon data. This work will present a dynamic representation of soil carbon where digital soil assessment can be easily incorporated.
- There is still very little forward looking on digital soil carbon assessment (Carré et al., 2007a). The maps of soil carbon have not been taken further for the assessments of soil functions and threats.
- A global map of soil carbon at a fine resolution with a consistent specification will help provide more information to decision makers, and modelers, and provide a better estimation of current carbon stock and distribution.

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CHAPTER TWO

Impacts of Changing Climate and Climate Variability on Seed Production and Seed Industry

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Contents

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Abstract

Agriculture is extremely sensitive to climate and weather conditions. The resilience of our crop production systems to changes in climate can be enhanced by improved understanding impacts and responses of crops to changing climates. Several countries in Asia and Africa are at the risk of losing about 280 million tons of potential cereal production as a result of climate change factor, particularly increasing temperatures and prolonged dry periods. The most significant negative changes for developing countries in Asia, where agricultural production declines of about −4% to −10% are anticipated under different socioeconomic and climate change scenarios. Rising temperatures will reduce the amount of fertile farmland, and by 2050, the amount of maize grown is expected to decline by 6–23% and wheat by 40–45%. The majority of the world's food supply comes from the consumption of seeds from grain crops (wheat, rice, maize, soybean, barley, and sorghum), which are most vulnerable to changing climates. The growth in food production is lower than the population growth; therefore, there will be challenges of food security. Major impacts of climate change will be on rain-fed crops that account for nearly 60% of cropland area. As predicted, South Asia and sub-Saharan Africa will be highly vulnerable to climate change. Crop production can be increased by the use of quality seeds of high-yielding stress-tolerant varieties, combined with judicious use of inputs, particularly water and nutrients. Climate changes affect all four dimensions of food security, that is, availability, access to food, stability of food supplies, and food utilization. The seed industry plays an important role in increasing food production. It provides high-quality seeds of high-yielding varieties in adequate quantities at the right time and right place. Climate change influences the population dynamics of insects, emergence of new pests, changing status of pest and disease development, and evolution of new races of pests. Quality seed production is also affected by crop/weed interactions, loss of pollinator biodiversity, and genetic diversity. The seed crop is also affected by climate change regarding change in crop phenology, reproduction, flowering, anthesis/pollen viability, and pollination/fertilization, length of seed-filling duration, seed setting, seed size, seed dormancy, seed yield, and ultimately seed quality.

Therefore, the cost of seed production is likely to increase in changing climate due to scheduling of operations, land and water management, herbicide/insecticide applications, pollination management, and postharvest seed management. Issues regarding intellectual property rights (IPR) related to seed, including patent infringements, prevalence of monocultures, consolidations of transnational corporations through acquisitions and mergers, and biodiversity and pollinator-loss related issues further complicate the problem. The lack of trained conventional plant breeders, crop physiologists, and seed technologists and stronger interdisciplinary collaboration between agronomists and biologists need attention. Further, acceptance of engineered crop or seed, increasing cost of genetically engineered (GE) seed as compared with conventional seed with no yield advantage, increasing number of herbicide applications, culminate in loss in net farm income in developed countries. In this article, the issues regarding the impact of climate change (particularly increasing temperature and carbon dioxide concentrations) on seed production, the present trend of the global seed industry, are discussed.



1. INTRODUCTION

The Intergovernmental Panel on Climate Change (IPCC) considers four families of socioeconomic development and associated emission scenarios, known as Special Report on Emissions Scenarios (SRES) A2, B2, A1, and B1 (Intergovernmental Panel on Climate Change, 2000). On the basis of the SRES emission scenarios, global mean surface temperatures are projected to increase from 1.8 °C (ranging from 1.1 to 2.9 °C for SRES B1) to 4.0 °C (ranging from 2.4 to 6.4 °C for A1) by 2100 (Intergovernmental Panel on Climate Change, 2007b). Another important change for agriculture is the increase in atmospheric carbon dioxide concentration [CO_2], which is projected to increase from ≈379 ppm today to >550 ppm by 2100 in SRES B1 to >800 ppm in SRES A1FI (Intergovernmental Panel on Climate Change, 2000, 2007b).

The world population reached the 7 billion mark in 2011. Eighty percent of this population currently resides in the less-developed regions. At the beginning of the century, this percentage was 70% (Agrawal, 2011). By 2050, the share of the world population living in the currently less-developed regions will have risen to 90%, and by that time, the world population is expected to cross the 9 billion (CGIAR, 2010). With the increase in population particularly in less-developed regions, demand for food will increase. The growth in food production is less than the population growth; therefore, there will be shortages of food causing food insecurity. Climate change SRES scenarios of A1 and A2 SRES suggest a likely increase in the disparities in cereal yields between developed and developing countries in a more significant way than has been found in previous studies (Parry et al., 2004).

Globally, the total land and total prime land would remain virtually unchanged at the current levels of 2600 and 2000 million hectares (ha), respectively. Pronounced regional shifts are expected with a considerable increase in suitable cropland at higher latitudes (developed countries +160 million ha) and a corresponding decline of potential cropland at lower latitudes (developing countries −110 million ha; Rosengrant and Sarah, 2003). Approximately 1,600 million ha of land are currently cultivated for crops (FAO, 2008a). Based on the Food and Agriculture Organization (FAO) estimates that, ignoring impacts on biodiversity and the carbon cycle, about 2400 million ha of land globally would be at least moderately suitable for wheat, rice, and grain maize cultivation, around 18% of the total world land area (FAO, 2000). Other studies have variously suggested between 50 million ha and 1600 million ha of land to be suitable for agricultural expansion (Delft, 2008).

Drylands cover 41% of the Earth's land surface and are home to >2 billion people, making up 35% of the world's population (Safriel and Adeel, 2005). More than 90% of dryland inhabitants are in developing counties, and half of the world's poor people live in drylands. They have the highest population growth rates of the world's major ecosystems, the lowest levels of human well-being, the lowest per capita income, and the highest infant mortality rates (Mearns and Norton, 2010). High temperatures cause much of the rainfall to be lost in evaporation; and the intensity of tropical storms means that much of the rain runs off, eroding the soil. Dryland areas experience substantial differences in rainfall, both within the year and between years. Rainfall also is highly variable over short geographic distances. Abiotic stress is the primary cause of crop loss worldwide, reducing the average yields for most major crop plants by >50% (Boyer, 1982; Bray et al., 2000). Ninety million people every year are affected by drought, 106 million people by flooding, and around 900 million ha of soil are affected by salinity. In addition, according to FAO estimates, the current annual loss worldwide to weeds is a staggering 95 billion US dollars. Of this, around 70 billion US dollars is lost in developing countries, equivalent to a loss of 380 million tons of wheat (Bruins, 2009). According to the International Fund for Agricultural Development (IFAD, 2001), 75% of the world's 1.2 billion poor live and work in rural areas.

Seed is one of the most crucial components in the livelihoods of agricultural communities. It is the repository of the genetic potential of crop species and their varieties resulting from the continual improvement and selection process over time. The potential benefits from increasing the use of quality seeds of a diverse range of crop varieties by farmers are widely

acknowledged as it increases food security through improved crop productivity (FAO, 2009). The seed industry plays an important role in increasing food production. It provides high-quality seeds of high-yielding varieties at the right time and right place. Crop production is increased by the use of quality seeds of high-yielding varieties and judicious use of fertilizers and water. It is estimated that, all other factors remaining the same, the use of quality seed of high-yielding varieties increases crop yield by 15–20% (Agrawal, 2011). In this article, the impact of changing climates (focusing of increasing temperature, dry periods, and carbon dioxide) on various components of the seed production and seed industry is considered. However, we recognize that other climate change factors such as change in precipitation, increased ultraviolet-B radiation, ozone pollution, and increasing greenhouse gases can also have a significant influence on seed production and industry; these factors are critically discussed.



2. CLIMATE CHANGE EFFECT ON CROP YIELD, FOOD SECURITY, AND NATIONAL GROSS DOMESTIC PRODUCT

According to the FAO, a temperature increase of 3–4 °C could cause crop yields to fall by 15–35% in Africa and West Asia and by 25–35% in the Middle East (FAO, 2008b). Several countries in Asia and Africa risk losing about 280 million tons of potential cereal production as a result of climate change (FAO, 2005). Europe experienced a particularly extreme climate event during the summer of 2003, with temperatures up to 6 °C above long-term means and precipitation deficits up to 300 mm. A record crop yield drop of 36% occurred in Italy for maize (*Zea mays* L.) grown in the Po valley where extremely high temperatures prevailed (Ciais et al., 2005). In a country such as India, major impacts of climate change will be on rain-fed crops including pulses that account for nearly 60% of cropland area (Basu et al., 2009).

The IPCC stated in its Fourth Assessment Report: "at lower latitudes, especially in seasonally dry and tropical regions, crop productivity is projected to decrease due to even small local temperature increases (1–2 °C), which would increase the risk of hunger" (IPCC, 2007). Fischer et al. (2005) projected the most significant negative changes for developing countries in Asia, where agricultural production declines of about −4% to −10% are anticipated under different socioeconomic and climate change scenarios.

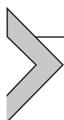
Asian rice (*Oryza sativa* L.) yields will decrease dramatically due to higher night-time temperatures coupled with generally warmer conditions.

According to the study conducted by the International Rice Research Institute (IRRI), rice yields are declining by 10% for every degree Celsius increase in night-time temperatures (IRRI, 2004). Recent temperature changes have been particularly marked, such that the warming trend in the last 50 years has been 0.13 °C decade^{-1}; nearly double that of the preceding 100 years. The yield of wheat declined by 5–8% according to Wheeler et al. (1996a), 10% Mitchell et al. (1993) per 1 °C rise in the mean seasonal temperature. In a global analysis of crop yields from 1981 to 2002, there was a negative response of wheat (*Triticum aestivum* L.), maize, and barley (*Hordeum vulgare* L.) yields to rising temperature, costing an estimated $5 billion per year (Lobell and Field, 2007). Old patterns of rainfall and temperature are shifting, due to more erratic weather, severe storms, and shifts in growing season lengths (Halweil, 2005). The IPCC report indicated that an overall increase of 2 °C in temperature and 7% in rainfall would lead to an almost 8% loss in farm level net revenue. In India, according to the National Climate Center in Pune, rainfall has decreased in July, and greater rainfall has been recorded in August in key crop-growing areas of the country. El Nino's Southern Oscillation phenomenon, with its associated cycles of droughts and flooding events, explains between 15 and 35% of global yield variation in wheat, oilseeds, and coarse grains (Ferris, 1999). Summerfield et al. (1984) observed lower grain yields with greater exposure to hot days (30–35 °C) during the reproductive period. Heat stress due to climate change at reproductive stage is increasingly becoming a serious constraint to chickpea (*Cicer arietinum* L.) production in northern India. In the US, maize and soybean (*Glycine max* L.) yields are predicted to decrease by 30–46% before the end of the century under the IPCC scenario with the slowest warming trend (Schlenker and Roberts, 2009).

In India, for a 2 °C increase in temperature, the reduction in the gross domestic product (GDP) is 5%, and for the next 6 °C, it would be 15–16%. In Haryana, wheat production has declined from 4106 kg ha^{-1} in 2000–2001–3937 kg ha^{-1} in 2003–2004, with the maximum temperature rising by about 3 °C during February–March in the last seven years (Gahukar, 2009). South Asia's prime wheat-growing land—the vast Indo-Gangetic plain, which produces about 15% of the world's wheat crop—will shrink 51% by 2050 due to hotter, drier weather and diminished yields, a loss that will place at least 200 million people at a greater risk of hunger (CGIAR, 2006). South Asia will be hit particularly hard by climate change as rising temperatures will reduce fertile farmland, and by 2050, the amount of maize grown is expected to decline by 6–23% and wheat by 40–45% (Anonymous,

2010a). A study conducted in India predicted that with an increase in temperature of up to 5 °C, the current wheat yield will come down by 38% even when technological growth is considered. The 1 °C increase in temperature with no associated CO_2 increase will lead to a decrease of 6 million tons of wheat. Increase in [CO_2] to 350 ppm is likely to reduce these losses by 4–5 million tons at all temperatures (Aggarwal and Swaroopa Rani, 2009). Similarly, a negative impact of climate change was observed in rice, maize, and sorghum (*Sorghum bicolor* L.). More negative impact was observed in rice, whose mean reduction in yield was predicted to be 6.7, 15.1, and 28.2% in 2020, 2050, and 2080, respectively (Agrawal, 2011).

Regional disparities around the global average impact are substantial. India and Africa are projected to see reductions of agricultural output by ≥30% (Cline, 2007). Generally, developing countries are more vulnerable to climate change because they depend heavily on agriculture, they tend to be relatively warm already, they lack the infrastructure to respond well to increased variability, and they lack capital to invest in innovative adaptations. Although Stern (2007) projected that a 2 °C increase in average temperatures would reduce world GDP by roughly 1%, the 2010 World Development Report of the Work Bank (2009) focuses on developing countries and estimates that without offsetting innovations, climate change will ultimately cause a decrease in the annual GDP of 4% in Africa and 5% in India (Lybbert and Sumner, 2010).



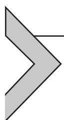
3. CLIMATE CHANGE EFFECT ON GEOGRAPHICAL DISTRIBUTION OF CROPS AND SPECIES COMPOSITION

Recent studies on the potential impacts of climate change on agriculture in the developing world offer a uniformly grim prognosis (ETC, 2008a). The immediate effects of rising temperatures mainly are concerned with the early onset of sexual reproductive development, higher or lower reproductive output, depending on the regions and species under study and the expansion of crop plants and shifts in geographic distribution of natural populations toward higher latitudes (Hedhly et al., 2008). Differential changes in phenology and growth between species in response to climate change could lead to new patterns of species coexistence during reproduction, potentially affecting competitive interactions and, ultimately, the species composition of the community (Chuine and Beaubien, 2001; Post et al., 2001). Climatic zones (and thus ecosystems and agricultural zones) could shift toward the poles by

150–550 km by 2100. Many ecosystems may decline or fragment and individual species may become extinct (Agrawal, 2011).

Twenty-three crops are projected to suffer decreases in suitable area, on average, while some 20 crops gain in suitable area. Overall, suitable area for crop cultivation is projected to increase. The biggest gains are in areas suitable for pearl millet (*Pennisetum glaucum* L., 31%), sunflower (*Helianthus annuus* L., 18%), common millet (*Panicum miliaceum* L., 16%), chickpea (15%), and soybean (14%), although many of the gains in suitable area occur in regions where these crops are currently not an integral component of food security. For example, land area suitable for pearl millet is projected to increase by >10% in Europe and the Caribbean, but these levels of increases are not projected for Africa, where the crop is currently widely cultivated (Lane and Jarvis, 2007). A study of wild plant species related to food crops estimates that 16–22% of the wild relatives of cowpea (*Vigna unguiculata* L.), peanut (*Arachis hypogaea* L.), and potato (*Solanum tuberosum* L.) will become extinct by 2055 and the geographic range of the remaining wild species will be reduced by more than half (CGIAR, 2007).

Arachis was the most-affected group, with 24–31 (depending on the migration scenario) of 51 species projected to go extinct and have their distribution area on average reduced by 85–94%, over the next 50 years. For *Solanum*, 7 (no migration) to 13 (unlimited migration) of 108 species were predicted to go extinct, and their range sizes reduce by approximately 38–69%. By region, Europe is projected to experience the largest gain in suitable areas for cultivation (3.7%). Olesen and Grevsen (1993) predicted that, for field-grown vegetable crops in Europe, increasing temperature will generally be beneficial, permitting an expansion of production beyond the presently cultivated areas. Antarctica and North America will also gain a suitable area of 3.2% and 2.2%, respectively. Sub-Saharan Africa and the Caribbean are projected to suffer a decline in land area suitable for cultivation of −2.6% and −2.2%, respectively. Models demonstrate a general trend of loss in suitable area in the Sahel, parts of Southern Africa, India, and northern Australia, and gains in the northern United States, Canada, and most of Europe (Lane and Jarvis, 2007).



4. CLIMATE CHANGE EFFECT ON INSECT PEST AND DISEASE DEVELOPMENT

Crop plants live in a very complex ecosystem. They live in competition with neighboring plants including weeds. Both are supported and/or

attacked by viruses, bacteria, fungi, insects, mites, spiders, amphibia, birds, and mammals. Damage by insect pests is usually caused by chewing on plant tissues or sucking the plant sap (e.g. aphids). In many cases, insect pests also transmit viruses, which then affect the plant. All these species interact with one another. Price (2002) estimated that globally there are 360,000 insect species that mainly live on plant material. Pimentel (2009) estimates that globally 70,000 pest species, including 9000 insects and mites, 50,000 plant pathogens, and 8000 species of weeds exist. About 10% of these 70,000 are considered to be major pests. Invasions by pests and pathogens have a huge impact on agriculture. Climate change can affect pathogen and pest dynamics in multiple ways. For airborne pathogen and pest organisms, higher temperatures may lead to faster disease cycles (Bouma, 2008). The effect of rising temperatures on insect pests and pathogens is reviewed briefly in the following sections.

4.1. Insect Pests

Temperature is one of the most important factor affecting insect ecology, epidemiology, and distribution, whereas plant pathogens are highly responsive to humidity and rainfall as well as temperature (Coakley et al., 1999). The relative importance of mean and extremes of temperature varies geographically. Insects respond to higher temperature with increased rates of development and with less time between generations. However, very high temperatures reduce insect longevity, whereas warmer winters reduce winterkill and consequently increase insect populations in subsequent growing seasons. Drought changes the physiology of host species, leading to changes in the insects that feed on them, and can reduce populations of friendly insects, such as predators or parasitoids, spiders, and birds (Rosenzweig et al., 2001). It has been estimated that with a 2 °C temperature increase insects might experience one to five additional life cycles per season (Yamamura and Kiritani, 1998). In general, higher temperatures increase the rate of development with less time between generations. Warmer winters will increase survival and possibly increase the insect populations in the subsequent growing season (Gutierrez, 2000; Rosenzweig et al., 2000). Models predict that recent warming trends are believed to have led to changes in winter survival and activity of insects (Crozier and Dwyer, 2006). It has long been known that elevation of atmospheric [CO_2] reduces the nitrogen/protein concentrations in most plants (Lincoln, 1993; Lincoln et al., 1986), and have a potential impact on human nutrition (Lieffering et al., 2004; Loladze, 2002), and can also affect

herbivores (Mattson, 1980) such as mites (Joutei et al., 2000) and insects (Fajer, 1989; Lincoln et al., 1986).

4.2. Insect–Pest Population Dynamics

Porter et al. (1991) pointed out that the effects of temperature on insects, including the limitation of geographical range, over wintering, population growth rates, number of generations per annum, crop pest synchronization, dispersal and migration, and availability of host plants. Global atmospheric [CO_2] has increased by approximately 30% in the last century and is believed to be responsible for an increase of 0.6 °C in the mean annual global surface temperature. If no climate policy interventions are made, the atmospheric [CO_2] may increase up to 405–460, 445–640, and 540–970 ppm by 2025, 2050, and 2100, respectively. Similarly, global mean temperature may increase by 0.4–1.1, 0.8–2.6, and 1.4–5.8 °C by the corresponding periods (Babu, 2011). Musolin (2007), for example, observed that in Japan, warmer climate led to the northward migration of the green stinkbug (*Nezara viridula* L.), a major agricultural pest damaging soybean, rice, cotton (*Gossypium* sp.), and many other crops. Further, his findings are consistent with those of Porter et al. (1991) that warming in temperate regions may have manifold effects on insects. The temperature increases associated with climatic changes could impact crop insect pest populations in several complex ways such as (a) extension of geographical range, (b) increased over wintering, (c) changes in population growth rate, (d) increased number of generations annually, (e) extension of development season, (f) changes in crop pest synchrony, (g) changes in interspecific interactions, (h) increased risks of invasions by migrant pests, and (i) introduction of alternative hosts and over regular hosts (Babu, 2011).

The geographical ranges of several important insects, weeds, and pathogens in the US have recently expanded, including soybean cyst nematode (*Heterodera glycines*) and sudden death syndrome (*Fusarium solani* f. sp. *glycines*) (Rosenzweig et al., 2000). Climate change could first affect disease directly by either decreasing or increasing the encounter rate between pathogen and host by changing rates of the two species (Bouma, 2008). In the nonirrigated paddy and wheat fields of Asia, the 1997 drought was followed by a particularly wet winter that delayed planting for two months in many areas and triggered heavy locust (*Locusta* sp.) and rat (*Rattus* sp.) infestations (World Watch Institute, 2011). Temperature may change gender ratios of some pest species, such as thrips (*Thrips* sp.), potentially affecting reproduction rates. Lower winter mortality of insects due to warmer

winter temperatures could be important in increasing insect populations (Harrington et al., 2001). Higher average temperature might result in some crops being able to be grown in regions further north; it is likely that at least some of the insect pests of these crops will follow the expanded crop areas. Insect species diversity per area tends to decrease with a higher latitude and altitude (Andrew and Hughes, 2005; Gaston and Williams, 1996).

4.3. Emergence of New Insect Pests and Change in Pest Status

Climate change is altering the distribution, incidence, and intensity of pests and diseases. Increases in temperature, variability in rainfall intensity and distribution, change in seasonality, drought, [CO_2] in the atmosphere, and extreme events affect pest dynamics in cropping systems. Any shift in the environmental temperature is likely to influence the behavior of insects more than that of plants and larger animals. In India in the past, gram pod borer (*Helicoverpa armigera*) has been a major pest of pigeonpea (*Cajanus cajan* L.) in most parts of the country. Now, the pod fly (*Melanogromyza abtusa*) is emerging as a serious pest of pigeonpea in central and south India. Similarly, the spotted pod borer (*Maruca vitrata*) has now become a major pest of short duration pigeonpea, both in north and central India (Ali et al., 2009).

Intensification of the hydrological cycle essentially means more droughts and floods, and more variable and extreme rainfall; similarly, longer growing seasons mean more generations of pests during the summer, whereas shorter and warmer winters mean that fewer adults, larvae, and eggs will die. Farmers in the tropics already find themselves near the temperature limits for most major crops, so any warming is likely to push their crops over the top (Halweil, 2005). In India, in general, sucking pests such as thrips, mites, jassids, and aphids are becoming problematic in recent years on different crops, as compared with lepidopterous larvae. Among the mites, leaf mite and panicle mite (sheath mite) on paddy rice and yellow mite on sugarcane (*Saccharum officinarum* L.) are some of the new problems. Another eriophyid mite (*Aceria caiani*) on pegionpea is transmitting sterility mosaic disease. Further, problems with unknown or complex etiology (such as parawilt of cotton) have taken serious proportions with the changing cropping systems/climatic patterns. Nematode problems have taken their toll, and with each passing year, some are gaining momentum to decimate the entire cropping systems. The root-knots, reniform, and burrowing nematodes are important in different states; *Meloidogyne indica* is devastating the Kagzi lime

(*Citrus latifolia* L.) in Gujarat, the root knot complex has similarly created serious situations on pomegranate (*Punica granatum* L.) in Maharashtra, Karnataka, and North Gujarat; the root knot is severely infesting mulberry (*Morus* sp.) plants, which is adversely affecting the silk industry in Karnataka, India (Babu, 2011).

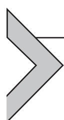
4.4. Disease Development

Crops can be damaged by diseases caused by fungi (rust, blight, mildew, and rot), bacteria/phytoplasma (wilt), and viruses. The occurrence of plant fungal and bacterial pests depends on climate and weather, and it is also strongly influenced by agricultural practices. Viruses and phytoplasma are often transferred via vectors, often insects (Weintraub and Beanland, 2006). Temperature, rainfall, humidity, radiation, or dew can affect the growth and spread of fungi and bacteria (Patterson et al., 1999). Other important factors influencing plant diseases are air pollution, particularly ozone and ultraviolet (UV)-B radiation (Manning and von Tiedemann, 1995) as well as nutrient (especially nitrogen) availability (Thompson et al., 1993). Elevated CO_2 may increase C_3 plant canopy size and density, resulting in a greater biomass with a much higher microclimate relative humidity. This is likely to promote plant diseases such as rusts, powdery mildews, leaf spots, and blights (Manning and von Tiedemann, 1995). Research on rice leaf blast and rice sheath blight in the temperate climes of Japan showed that elevated CO_2 increased the potential risks for infection from leaf blast and epidemics of sheath blight (Neumeister, 2010).

Temperature, precipitation, humidity, dew, radiation, wind speed, and circulation patterns influence the growth, spread, and survival of crop pathogens. Increased temperature and humidity result in the spread of diseases as wet vegetation promotes the germination of spores and proliferation of fungi and bacteria. Some pathogens (e.g. powdery mildews) thrive in hot, dry conditions as long as there is dew formation at night. Climate conditions also influence postharvest pest damage. Beneficial and harmful insects, microbes, and other organisms in the environment will also be responding to the changes in [CO_2] and climate (Goho, 2004). Host plants such as wheat and oats (*Avena sativa* L.) become more susceptible to rust diseases with increased temperatures, but some forage species become more resistant to fungi with increased temperature (Coakley et al., 1999). Temperature and precipitation changes can impact insect pest predators, parasites, and diseases resulting in complex dynamics. Fungal pathogens of insects are favored by high humidity, and their incidence would be increased by climate changes

that lengthen periods of high humidity and reduced by those that result in drier conditions (Babu, 2011). Gangopadhyay and Mathur (1978) observed that the intensity of mycoflora on vegetables seeds in Kullu Valley, Himachal Pradesh (India) reported 96% infection of *Alternaria brassicicola* on cabbage (*Brassica oleraca* L.) seed and 100% infection of *Verticillium* sp. in brinjal (*Solanum melongena* L.). Further, capsicum (*Capsicum* sp.) seeds were found to be infected up to an extent of 80% with *Verticillium* sp., 68–80% with *Fusarium moniliforme*, and 63% with *Colletotrichum* sp. In light of the above observations, the problem is likely to be aggravated further (Kumar et al., 2009). In 1999, a new strain of wheat stem rust (*Puccinia graminis*) appeared in eastern Africa. It is able to overcome resistance in many of the world's most widely sown wheat varieties. Rust spores of this strain travel over huge distances, so it is only a matter of time before the new rust race infects PBW343, a susceptible wheat variety sown on some 8 million ha in India.

Increasing pest and pathogen load due to congenial conditions occurs: an increase in the temperature accompanied by changing vapor pressure deficits that result in altered relative humidity in the canopy region of the crop creates conditions favorable for the insect pest and disease-causing pathogens to parasitize on the crop at a frequency and intensity higher than normal. An example of such a scenario is the epidemic-like situation of yellow rust (*Puccinia* sp.) severity on variety PBW343 in Punjab in February 2009, and unprecedented infestation by brown plant hopper (*Nilaparvata lugens*) on rice varieties in Kharif 2008 in Punjab and Haryana. Such an adaptation by a race of yellow rust was never before noticed in such temperature situations. Such consequences are unpredictable and likely to occur with many other pathogens and pests (Gupta, 2009).



5. CLIMATE CHANGE EFFECT ON C_3/C_4 CROPS AND CROP/WEED INTERACTIONS

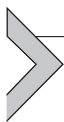
Weeds compete with crops for soil nutrients, light, and space. Drought conditions increase competition for soil moisture. The effect of climate change varies on different crop species as some will have more pronounced effects as compared with others, depending on their photosynthetic pathway. C_4 plants account for a small fraction of the total number of plant species [(<1000 out of 250,000) (Elmore and Paul, 1983)]. According to Holm et al. (1977), 14 of the world's worst weeds are C_4 plants, whereas around 76% of the harvested crop area in 2000 was grown with C_3 crops (Monfreda et al., 2008). Optimal temperatures for growth in C_4 plants are generally

higher than optimal temperatures for C_3 plants (Flint and Patterson, 1983), but with higher [CO_2], the optimum temperature of many C_3 plants also increases (Bunce and Ziska, 2000). The benefit of elevated [CO_2] under sufficient water conditions will lead to a higher C_3 weed competitiveness in C_4 crops (Neumeister, 2010). However, C_4 crops might outcompete better growing C_3 weeds in drought situations and at higher temperatures. Some plant species can effectively use mycorrhiza and compete with crops (Tang et al., 2009). Same type of plants (with regard to photosynthesis) in the same ecosystem would react to changes in a similar way. This is only partly true; as C_3 crops and C_3 weeds both benefit from elevated [CO_2]; however, the magnitude of responses can differ. An experiment that investigated seven C_3 crop and three C_3 weeds at 350 and 700 ppm CO_2 showed similar growth rates and mass of C_3 crops and C_3 weeds (Bunce, 1997). Because all C_4 plants (weeds and crops) have the same photosynthesis path, they may react to changes in the same ecosystem in a similar fashion.

The benefit of elevated [CO_2] under sufficient water conditions will lead to a higher C_3 weed competitiveness in C_4 crops. Most C_3 weeds benefit more than C_3 crop species from elevated CO_2 (Ziska, 2003). Wand et al. (1999) reported that with no stresses, elevated CO_2 decreased stomatal conductance by 39 and 29% for C_3 and C_4 species, respectively. In addition to soybean, the yield of the C_3 crops such as wheat, oats, snap bean (*Phaseolus* sp.), pepper (*Capsicum* sp.), rape (*Brassica* sp.), and various cucurbits are highly sensitive to chronic ozone exposure. C_4 crops are generally much less sensitive to ozone. Many weeds respond more positively to increasing [CO_2] than do most cash crops, particularly C_3 "invasive" weeds that reproduce by vegetative means (e.g. roots, stolon, etc.) (Ziska and George, 2004). Although many weed species have the C_4 photosynthetic pathway, and therefore show a smaller response to atmospheric CO_2 relative to C_3 crops, in most agronomic situations, crops are in competition with a mix of both C_3 and C_4 weeds. For all weed/crop competition studies where the photosynthetic pathway is the same, weed growth is favored as CO_2 is increased (Ziska and Runion, 2006). The loss of CO_2 in cotton under photorespiration is 30–40%. It is connected with the fact that C_3 plants do not possess the mechanism of [CO_2] enrichment as in maize and sorghum. On average, across several species and under unstressed conditions, crop yields are expected to increase in the range of 10–20% for C_3 crops and 0–10% for C_4 crops (Ainsworth et al., 2004; Gifford, 2004). However, temperature and precipitation changes in future decades will also modify, and potentially limit, direct CO_2 effects on plants (IPCC, 2007).

Competition occurs between C_3 crop and C_4 weed species under different climate and [CO_2] (Ziska and George, 2004). The [CO_2]-temperature interactions are recognized as a key factor determining plant damage from growth, yield and pest infestation in future decades; [CO_2]/precipitation interactions will likewise be important. Most studies continue to investigate pest damage as a separate function of either [CO_2] (Agrell et al., 2004; Chen et al., 2005) or climate, mostly temperature. Increases in [CO_2] will likely stimulate the growth of weeds. Some weeds respond more positively to increasing [CO_2] than do most crops, particularly C_3 "invasive" weeds that reproduce by vegetative means (Ziska and George, 2004).

Therefore, the response and interaction of C_4 weeds in C_3 crops, C_3 weeds in C_4 crops, and performance and interactions of weeds and crops of similar photosynthetic pathway, that is, C_3 weeds in C_3 crops and C_4 weeds in C_4 crops need to be further investigated. Similarly results based on simulation need to be confirmed with field performance. Moreover, under most of the simulation studies, either of the two important factors responsible for climate change (temperature and CO_2 concentration) taken at the same time and the interaction between the two and weeds and crops is presently lacking.



6. CLIMATE CHANGE EFFECT ON SEED PRODUCTION

6.1. Crop Phenology

The effect of global climate change on plant phenology has been extensively reviewed (Cleland et al., 2007). Phenology is a sensitive biosphere indicator of climate change. Plant phenology has been advanced by 2–3 days in spring and delayed by 0.3–1.6 days in autumn per decade (Myneni et al., 1997; Parmesan and Yohe, 2003). In Germany, the phenology of 78 agricultural and horticultural events between 1951 and 2004 was, on average, 1.1–1.3 days earlier decade^{-1} (Estrella et al., 2007). Chmielewski et al. (2004) found that in Germany, for the period 1961–1990, the beginning of the growing season advanced by 2.3 days decade^{-1}, following increases in the mean annual air temperature of 0.36 °C per decade. Over the same period, warmer temperatures advanced the beginning of stem elongation in rye (*Secale cereale* L.) by 2.9 days decade^{-1}; the beginning of cherry tree blossom (*Prunus* sp.) by 2 days decade^{-1}; and the beginning of apple tree (*Malus domestica* L.) blossoming by 2.2 days decade^{-1}.

Flowering and fruiting phenology of plants is very sensitive to environmental cues such as temperature, moisture, and photoperiod; thus, it

is imperative to understand the impact of climate change on reproductive phenology. Phenological differences in reproductive events among species over the growing season may reduce competition by spreading primary resource use over different temporal pools (Henry et al., 2001).

Abrol and Ingram (1996) emphasized that increased temperature would affect the crop calendar in tropical regions. In the tropics, however, rising temperature is likely to reduce the length of effective growing season, particularly where more than one crop per year is grown. Sustained temperature increases over the season will change the duration (from sowing to maturity) of the crop (Roberts and Summerfield, 1987), while short episodes of high temperature at critical stages of crop development can impact yield independently of any substantial changes in the mean temperature (McKeown et al., 2005; Wheeler et al., 2000). Record high daytime and night-time temperatures over most of the summer growing season reduced leaf and grain-filling development of key crops such as maize, fruit trees, and vineyards; accelerated crop ripening and maturity by 10–20 days and resulted in reduced soil moisture and increased water consumption in agriculture (Easterling et al., 2007). Kudo et al. (2004) reported that high temperatures can change a plant's phenology—the annual timing of bud break, flowering, seed production, and other things linked to seasonal climate change. It can also alter the number, size, and orientation of leaves and might change the depth of plant roots. High temperatures are likely to shorten the growing cycle of many crop species and, during some developmental stages such as the reproductive phase, most crops are only able to tolerate narrow temperature changes, which, if exceeded, can reduce seed set and thus yield (Porter, 2005). For biological and agricultural systems, the timing and frequency of extreme temperature events could be more important than the increase in the mean temperatures (White et al., 2006).

6.2. Reproduction

Heat stress is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature. The effect of heat stress in many plant species is induced sterility when heat is imposed immediately before or during anthesis (Prasad et al., 2000, 2008). Pulse legumes are particularly sensitive to heat stress at the bloom stage; only a few days of exposure to high temperatures (30–35 °C) can cause heavy yield losses through flower drop or pod abortion (Prasad et al., 1999; Siddique et al., 1999). Studies of long-term elevated temperature showed that reproductive processes are adversely affected much sooner than vegetative processes for rice

(Baker et al., 1995), soybean (Boote et al., 2005), common bean (*Phaseolus vulgaris* L.; Prasad et al., 2002), peanut (Prasad et al., 2003), cotton (Reddy et al., 2000), and sorghum (Prasad et al., 2006a). The staple cereal crops can tolerate only narrow temperature ranges which, if exceeded during the flowering phase, can damage fertilization and seed production, resulting in reduced yield (Porter, 2005). Soybean, peanut, and cotton have several weeks for the success of reproductive structures. Rice and peanut have the same sensitivity of grain yield, seed harvest index (HI), pollen viability, and success in grain formation in which pollen viability and percent fertility at air temperature >33 °C and reaches zero at 40 °C.

Successful fruit set depends on several reproductive processes including pollen germination and tube growth processes. Sexual reproduction in plants is more sensitive to high temperatures than are vegetative processes, and therefore, plant reproductive organs are more vulnerable to changes in short episodes of high temperatures before and during early flower stage (Prasad et al., 2000, 2001; 2008; Reddy and Kakani, 2007). High temperatures accelerate overall female development. Thus, high temperatures accelerate stigma and ovule development, reducing the duration for which they are receptive to pollen and pollen tubes. Consequently, temperature stress seems to have a complementary effect on the male and female counterparts, accelerating or slowing down the reproductive process. This might have severe consequences for the reproductive output, inducing low seed and/or fruit set (Hedhly et al., 2007). Male–female synchrony seems to be a prerequisite for successful mating in plants (Herrero, 2003), and fertilization occurs when the two gametes are at the same phase of the cell cycle (Friedman, 1999). The base and optimal temperatures for vegetative growth, reproduction, and yield with regard to important field crops are given in Table 2.1 and for germination, growth, and yield in vegetable crops in Table 2.2 (Hatfield et al., 2008).

6.3. Flowering

The timing of flowering within a season is largely determined by responses to temperature and photoperiod, and in plants at suboptimal temperatures, these quantitative responses are reasonably well understood (Wallace and Yan, 1998). In recent years, molecular biology has also greatly contributed to our understanding of flowering gene pathways (Baurle and Dean, 2006; Tsuji et al., 2008). Menzel et al. (2006) reported that 78% of all observations in 21 European countries showed earlier flowering, with an advance in phonological events of 2.5 d per decade on average. The yield of field-grown

Table 2.1 Cardinal, Base, Optimum, and Ceiling, Temperatures for Vegetative and Reproductive Processes of Selected Crops

	Base, optimum, and optimum range temperature for vegetative processes			Base, optimum, optimum range, and failure temperatures for reproductive processes			
Crop	**Tbase, °C**	**Topt, °C**	**Topt range, °C**	**Tbase, °C**	**Topt, °C**	**Topt range, °C**	**Failure, °C**
Maize	8	34	25–37	8		18–22	35
Soybean	7	30	20–30	6	25–37	22–24	39
Wheat	0	26	33	1	20–30	15	34
Rice	8	36	26–34	8	33	23–26	35–36
Sorghum	8	34	34	8	26–34	25	35
Cotton	14	37	31–35	14	34	25–26	35
Peanut	10	>30	23	11	31–35	20–26	39
Bean					23	24–24	32
Tomato	7	22	25–37	7		22–25	30

Adapted from Hatfield et al. (2008).

canola (*Brassica* sp.) was reduced significantly by temperatures of ≥30 °C during flowering. The yield of three *Brassica* species, *B. napus*, *B. rapa*, and *B. juncea* was all reduced by high-temperature stress, although reductions were less in *B. rapa* and *B. juncea* than in *B. napus* (Angadi et al., 2000).

Indeterminate crops undergo floral initiation over a longer period of time and floral development, and events coinciding during nonstress or lower stress periods can compensate for inhibited development during the periods of higher stress (Prasad et al., 2008). Among pulses, pigeonpea is very sensitive to abrupt fluctuations of temperatures, with either lower or higher extremes leading to massive flower drop. At extreme high temperature, a serious adverse impact was reported on pod setting. For cool-season pulse crops, daily maximum temperature >25 °C is considered as the threshold level for heat stress, as it affects seed yield by reducing flowering, fertilization, and seed formation. A brief exposure to high temperature during flowering causes heavy loss due to flower drop and pod abortion, and during filling, it can reduce seed set and seed weight and can accelerate senescence, resulting in reduced seed yield (Ali et al., 2009). Exposure to heat stress during flowering results in pollen sterility and loss of seed-set in legumes (groundnut, Prasad et al., 2000; dry bean, *P. vulgaris* L., Prasad et al., 2002; cowpea, *V. unguiculata* (L.) Walp., Ahmed et al., 1992; soybean, Salem et al., 2007; Djanaguiraman et al., 2010, 2011) and cereals (rice, Jagadish et al., 2007; Prasad et al., 2006b; Satake and Yoshida, 1978; wheat, Saini et al.,

Table 2.2 Temperature Thresholds for Various Growth Processes of Selected Vegetable Crops

Climatic classification	Crop	Acceptable temperature (24 °C) for germination	Optimum temperature (°C) for yield	Acceptable temperature (°C) range for growth	Frost sensitivity
Hot	Watermelon	21–35	25–27	18–35	+
	Okra	21–35	25–27	18–35	+
	Melon	21–32	25–27	18–35	+
	Sweet potato	21–32	25–27	18–35	+
Warm	Cucumber	16–35	20–25	12–30(35)	+
	Pepper	16–35	20–25	12–30(35)	+
	Sweet corn	16–35	20–25	12–30(35)	+
	Snap bean	16–30	20–25	12–30(35)	+
	Tomato	16–30	20–25	12–30(35)	+
Cool–warm	Onion	10–30	20–25	7–30	−
	Garlic		20–25	7–30	−
	Turnip	10–35	18–25	5–25	−
	Pea	10–30	18–25	5–25	(−)
Cool	Potato	7–26	16–25	5–25(30)	+
	Lettuce	5–26	16–25	5–25(30)	(+)
	Cabbage	10–30	16–18(25)	5–25	−
	Broccoli	10–30	16–18(25)	5–25	−
	Spinach	4–16	16–18(25)	5–25	−

Adapted from Hatfield et al. (2008).

1983). Flower abortion also has been attributed to the decreased seeds per plant and seed yield in other crops such as *B. napus* (Angadi et al., 2000), *B. rapa* (Morrison and Stewart, 2002), and *B. juncea* (Gan et al., 2004).

6.4. Anthesis/Pollen Viability

Higher temperature during anthesis increased the sterility of the pollen grains in rice. The maximum sterility of 21.5% was observed at 34.3 °C. The maximum pollen germination was observed when the mean temperature during anthesis was about 20 °C. But pollen germination decreased above 32 °C. In wheat, low temperature during anthesis was deleterious to pollen viability and fertilization. The maximum sterility of 43% was observed at a low mean temperature of 12.9 °C during anthesis. Pollen germination on stigma was increased with increasing temperature. Maximum pollen germination of about 85% was observed at a temperature range of 17–22 °C. At higher temperatures (>23 °C), pollen germination was reduced (Agrawal,

2011). Heat stress affects male and female gametogenesis, pollen–pistil interaction and embryo development. The three processes interact and have a bearing on each other (Hedhly et al., 2008). In maize, a highly compressed phase of anthesis exists, whereas spikelets on rice and sorghum may achieve anthesis over a period of a week or more. Air temperatures >36 °C cause pollen to lose viability in corn and in postblooming reduce grain yield in soybean. Impairment of pollen and other development by elevated temperatures is another important factor contributing to a decreased fruit set in many crops at moderate to high temperatures (Sato et al., 2006). However, even small increases in temperature above the optimum can very negatively affect pollen viability via a range of mechanisms that lead to yield penalties (Hedhly et al., 2008). It is even possible that high temperature stress may cause altered genetic frequencies of the subsequent sporophytic generation (Hormaza and Herrero, 1994), thereby possibly forwarding consequences to future generations. Within a permissive range, warming temperatures accelerate both the rate of pollen tube growth as well as stigma and ovule development, thus maintaining the male–female synchrony necessary for successful seed set. However, under high-temperature stress, this synchrony can be lost, leading to lower fertility and yield reduction (Hedhly et al., 2008). Pollen development, fertilization, and asynchrony of stamen and gynecium development are sensitive to temperatures during flowering (Boote et al., 2005). The pollen loss or stigma viability under high temperatures stress might be the primary reason for the lowered number of seeds produced in legumes (Davies et al., 1999; Hall, 2004; Srinivasan et al., 1998). A significant negative correlation between pollen production and temperature was reported in groundnut (Prasad et al., 1999). A lower seed yield at high temperature under both ambient and elevated [CO_2] conditions was shown to be due to decreased pollen viability in groundnut and bean (Prasad et al., 2002, 2003). Pollen sterility and pollen production at high temperatures may also be associated with the early degeneration of the tapetal layer of pollen (Porch and Jahn, 2001).

Pollen grains once released from anthers act as independent functional units and are exposed to ambient environment. Therefore, episodes of high temperature during flowering would more severely affect pollen than the deeply seated ovules. Studies have shown that microsporogenesis and megasporogenesis are injured by high temperature, resulting in reduced fruit set (Cross et al., 2003; Young et al., 2004), and also suggest that pollen plays a major role in fruit set under high temperature conditions. This reduction in time from anthesis to maturity at high temperatures has been attributed to

an inability of synthetic processes in the grain to respond to temperature, rather than the effect on the availability of assimilates (Bhullar and Jenner, 1986; Wardlaw et al., 1980). Lower seed set under heat stress can be caused either by poor anther dehiscence causing low numbers of germinating pollen grains on the stigma (Jagadish et al., 2007), or because of decreased pollen viability (Prasad et al., 2000, 2002, 2006a, 2006b) or ovule function (Gross and Kigel, 1994). Both microsporogenesis (pollen development) and megasporogenesis (stigma development) are injured under heat stress, resulting in lower seed-set (Cross et al., 2003; Young et al., 2004). Pollen is known to be relatively more sensitive to heat stress conditions.

6.5. Pollen Germination

Temperature has a clear effect on pollen tube kinetics, expressed as the time required for pollen germination and the rate of pollen tube growth. Although temperature affects pollen tube kinetics, information on the effect of temperature on pollen tube dynamics, expressed as the census of the microgametophyte population that succeeded in reaching the base of the style, is lacking (Hedhly et al., 2004). Pollen germination and rate of pollen tube growth are also highly sensitive to heat stress (Kakani et al., 2002; Prasad et al., 2000, 2001). Short periods of heat stress can also influence pollen viability, seed set, and grain growth (Prasad et al., 2000, 2001; Stone and Nicolas, 1998). As such, short durations of temperature stress can cause sterility; the timing of the episode of the high temperature relative to peak flowering is reported to be very critical to quantify the impact of heat stress (Wheeler et al., 2000). The quantity and morphology of pollen, anther dehiscence, and pollen wall architecture as well as the chemical composition and metabolism of pollen (Koti et al., 2005; Prasad et al., 2002) have been shown to be affected by high temperatures. All these effects could alter male fitness by reducing the available amount of pollen and could also—if the pollen limitation is severe—indirectly limit female fitness by reducing the number of seeds sired. Mild increases in temperature negatively affect characteristics such as pollen viability (Aloni et al., 2001), pollen germinative ability (Koti et al., 2005), pollen tube growth rate (Johannsson and Stephenson, 1998), and seed and fruit set (Sato et al., 2002). High temperature inhibits pollen germination and pollen tube growth and genotypes differ in their sensitivity (Huan et al., 2000; Kakani et al., 2002). Plants exhibit greater reproductive survivability at extreme (low and high) temperatures normally encountered during plant reproduction and for processes leading to yield such as pollen grain development,

pollen germination, pollen tube growth, fertilization and embryo development, and finally seed development.

6.6. Length of Seed/Grain Filling Duration

A brief exposure of plants to high temperatures during seed filling can accelerate senescence, diminish seed set and seed weight, and reduce yield (Siddique et al., 1999). The acceleration of the vegetative cycle can have negative effects on grain filling and quality. Grain- or seed-filling duration is the time from seed set to physiological maturity. Postflowering conditions affect the yield, mainly by influencing ovary or seed abortion, or by changing seed-filling duration or seed-filling rate. In general, seed-filling duration is more plastic than seed-filling rate. Despite the importance of individual seed size, yield variation is more often due to changes in seed number than to individual seed mass at maturity. Moderate increase in temperatures hastens crop development and therefore shortens the seed-filling duration. Seed number can be reduced via the direct effects of high temperature on reproduction, particularly pollen formation and function. Because warming speeds reproductive development, the seeds that do develop are often small. Although seed filling rate is sometimes stimulated by warming, this effect often does not fully compensate for shortened seed-filling duration (Thuzar et al., 2010). Both drought (de Souza et al., 1997) and heat stress (Prasad et al., 2006a) decrease the seed-filling duration and thus lead to smaller seeds. The impact of heat stress on seed-filling rates and seed-filling duration is similar to that of drought. However, there may be a slight increase in the seed-filling rate but a large decrease in seed-filling duration under heat stress. The increase in seed-filling rate does not compensate for the loss of duration, thus resulting in smaller seed size and seed yields (Shipler and Blum, 1986; Tashiro and Wardlaw, 1989). The yield of cereals decreases in proportion to the shortening of the grain-filling period as temperature increases. For example, temperatures as low as 25 °C can reduce the grain-filling period in wheat, after which a 1 °C temperature rise shortens the reproductive phase by 6%, shortens the grain-filling duration by 5%, and reduces grain yield and HI proportionally (Lawlor and Mitchell, 2000).

Heat stress, singly or in combination with drought, is a common constraint during anthesis and grain-filling stages in many cereal crops of temperate regions. Temperatures >30 °C in maize reduced grain sink strength and yield. As the mean temperature increases above 23 °C, seed growth rate, seed size, and seed HI in soybean decrease until reaching zero at a mean temperature of 39 °C (Thomas, 2001). Baker et al. (1989) reported that the

progressive reduction in seed size at >23 °C, along with reduction in fertility at >30 °C, results in the reduction in seed HI at temperatures >23 °C or >27 °C, reaching zero HI at 39 °C (Boote et al., 2005). Lawlor and Mitchell (2000) reported that a 1 °C rise would shorten reproductive phase in wheat by 6% and grain-filling duration by 5% and reduce grain yield and HI proportionately. Bender et al. (1999) analyzed spring wheat grown at nine sites in Europe and found a 6% decrease in yield per 1 °C temperature rise. Lobell and Field (2007) reported a 5.4% decrease in the global mean wheat yield per 1 °C increase in temperature. Laing et al. (1984) found the highest bean yield at 24 °C, with a steep decline at higher temperatures.

Cool season pulses such as chickpea, lentil (*Lens culinaris* L.), and lathyrus (*Lathyrus* sp.) have a reasonably high tolerance to heat, which enables them to set pods but filling of pods is seriously jeopardized at high temperature and leads to low productivity (Ali et al., 2009). Greater reductions were observed with longer duration of exposure to high temperatures, especially during seed fill and maturation (Prasad et al., 2008). Also, high temperature stress during reproductive development may have negatively affected cell expansion, cotyledon cell number, and thus seed-filling rate, resulting in lowered weight per seed (Munier-Jolain and Ney, 1998).

6.7. Seed Size

Zavitz (1910) argued that farmers should plant only the larger seeds of a kind or variety for crop production. Similarly, Kiesselbach (1924) demonstrated a relationship between seed size and productivity in the small grain crops and advocated sieving to eliminate the smaller seeds before planting. Density is the physical property most consistently associated with seed germination and vigor (Clark, 1904; Delouche, 1986; Whitcomb, 1936). Seed size and density interact with physiological quality and performance potential. The small-light seeds are lowest in quality, whereas the large-heavy seeds (excluding the very heaviest and very largest) are the highest in quality. It is already well known that seed lots of certain crop kinds with reduced quality caused by weathering (e.g. soybean, sorghum, cowpea) drought-induced immaturity (e.g. wheat), and immaturity due to indeterminacy of late tillering (e.g. cotton, peanut, rice) are especially suited for upgrading through density grading (Delouche et al., 1995).

Heat stress increased the percentage of shriveled seed and decreased seed size (Prasad et al., 2003). Seed protein concentration of soybean has been shown to increase at temperatures >25 °C (Piper and Boote, 1999). Oil and protein concentrations were inversely related in response to heat stress

during seed fill (Piper and Boote, 1999). In contrast, quantifying the effects of temperature on oil and protein concentrations of soybean seed cultured in vitro, Pipolo et al. (2004) concluded that both protein and oil concentrations were positively related and were a function of rate of dry matter accumulation of soybean seed. Therefore, temperature influence on seed size may result from changes in overall seed-growth rates, which in turn are likely to be dependent on carbon and nitrogen supply to the seeds (Pipolo et al., 2004).

In soybean, as temperature increased to >26/20 °C, weight per seed decreased (Baker et al., 1989). Temperatures >30/25 °C during flowering and pod development reduced weight per seed, regardless of the temperature during seed fill (Egli and Wardlaw, 1980). Temperatures >29/20 °C during seed fill decreased soybean weight per seed (Dornbos and Mullen, 1991). The duration of grain growth is shortened at high temperatures, but the effect of this reduction is not necessarily compensated for entirely by an increase in the rate of grain growth, thereby resulting in reduced grain size (Tashiro and Wardlaw, 1989; Wardlaw et al., 1980). Grabe (1980), however, pointed out that many farmers still save seeds of small grain crops, such as wheat, for planting, and they could significantly improve yields in many cases by keeping only 25–30% of the largest seeds for sowing and marketing the rest with little if any extra cost.

6.8. Seed Yield

Studies showed that 25 °C was the typical optimum temperature for grain yield of rice, soybean, and peanut and that the yield declined progressively as temperature increased, causing complete failure at 35 °C for rice and sorghum, and at 39–40 °C for peanut and soybean (Boote et al., 2005). Similarly, in rice, Peng et al. (2004) reported that grain yield declined by 10% for each 1 °C increase in growing-season minimum temperature in the dry season; according to the researchers, their report provides direct evidence of decreased rice yields from increased night-time temperature. In the past century, the daily minimum night temperature increased at a faster rate than did daily maximum temperature in association with a steady increase in atmospheric greenhouse gas concentrations (Easterling et al., 1997). Yields increased with temperature up to 29 °C for corn, 30 °C for soybeans, and 32 °C for cotton, but temperatures above these thresholds are very harmful. Holding current growing regions fixed, area-weighted average yields are predicted to decrease by 30–46% before the end of the century under the slowest (B1) warming scenario and to decrease by 63–82% under the most

rapid warming scenario (A1FI) under the Hadley III model (Schlenker and Roberts, 2009). Lonbell and Asner (2003) reported that in maize and soybean production relative to climatic variation in the United States, there was a 17% reduction in yield for every 1 °C rise in temperature because of the confounding effect with other yield-limiting factors. In a recent evaluation of global maize production response to both temperature and rainfall over the period 1961–2002, Lobell and Field (2007) reported an 8.3% yield reduction per 1 °C rise in temperature.

Gan et al. (2004) found that the seed yield of canola decreased by 15% when high temperature stress was applied before flowering, whereas the yield reduction was 58% when the stress was delayed to the period of flowering, and further to 77% when the stress was delayed to the pod developmental stage. Physiologically, high temperature stress during reproductive development may have affected flower abortion, subsequent sink size, and later pod abscission, resulting in a decreased number of seeds per plant (Duthion and Pigeaire, 1991). Such effects have already been felt in the seed industry of India. Because seed production requires a certain degree of chilling to induce seed formation in temperate crops, many vegetable seed farms are located in mountainous regions, such as the Hindu Kush Himalayas. Although mountainous regions can provide such a climate, they also make farmers increasingly vulnerable to the effects of climate change. Farmers in the Kullu valley of Himachal Pradesh state in India are finding that overall temperatures have been rising, while rains have become more unpredictable, leading to several crop failures. Cole crop seed yields have been decreasing, yet the challenge of ensuring sufficient natural pollination under changing climatic conditions has not been addressed by researchers, much less farming communities (Sharma, 2006). Gill and Singh (1973) reported that in the north-western Himalayan region of India, fluctuation in temperature in February when curds are formed did great damage and reduced the seed yield by 49–100%. Heavy rainfall and snowfall in January and February were very harmful to seed production. They suggested that seed production was better at lower altitudes (1200–1450 m). Reproductive growth leading to seed yield is often depressed by the same increase in temperature that enhances vegetative growth and development. In soybean, at a temperature of 35 °C for 10 h during the day, yield reductions of about 27% were measured (Gibson and Mullen, 1996).

In the upper Kullu Valley in the Hindu Kush Himalayas, the maximum temperature of the pod setting period (May) increased by 1.58 °C. The minimum temperature for April (March–April is bolting and flowering

period) and August increased by 2.03 and 2.16 °C, respectively. An increase in temperature adversely affected seed production. In the case of cabbage, a reduction in seed yield of about 15–43% over a period of about 24 years was observed (Kumar et al., 2009).

6.9. Seed Dormancy

In commercial seeds, dormancy is not only a problem for plant establishment but is also a problem during seed quality evaluation. Because the objective of the standard seed germination test is to report germination percentage under optimal conditions, seed analysts must identify treatments to overcome dormancy and favor germination when dormancy exists in a seed lot. Environmental factors affecting seed dormancy include temperature, water availability, light quality, photoperiod, altitude, and mineral nutrition. Although the response to each factor will be influenced by the kind of seed dormancy, some trends have been observed. For example, high temperature, drought, and short days during seed development are usually associated with higher germinability or lower seed dormancy intensity. High temperatures during seed development are generally associated with lower physiological dormancy, which may be caused by reduced synthesis of inhibitory compounds (e.g. abscisic acid) at high temperatures, or greater synthesis of promoting substances (e.g. gibberellins). However, there are also examples of species in which dormancy increases with higher temperatures during seed development; for instance, high temperatures caused the development of thicker soybean seed coats resulting in more dormant seeds. In this case, the same factor (temperature) affects dormancy differently depending on the dormancy mechanism (physiological versus physical). The type of response to drought conditions during seed development is also influenced by the kind of dormancy. When dormancy is imposed mechanically by a thick seed coat, drought usually increases its thickness, thereby contributing to reduced germinability. In contrast, drought typically causes a reduction in seed dormancy when it is imposed biochemically, possibly by interfering with the synthesis of inhibitors or promoters of germination. With respect to the light environment of the maternal plant, day length and light quality (wavelength composition) influence germinability during seed development (Contreras et al., 2008).

6.10. Seed Quality

There are three important aspects of seed quality: (i) size of individual seed, (ii) composition or nutritional quality of seed, and (iii) ability of seed to

germinate and grow. Growth environment plays an important role in all three aspects of seed quality. Drought and heat stress can have a profound impact on seed quality of cereals and legumes, mainly because of their impact on nutrient uptake, assimilate supply, partitioning, and remobilization of nutrients (Prasad et al., 2008). Much of the variation in seed quality among seed lots is the direct or indirect result of variation in weather before or at harvest, hot dry periods generally providing good quality seed (Austin, 1972). High temperatures after anthesis have been shown to reduce seed quality in bean (*P. vulgaris* L.; Siddique and Goodwin, 1980) and rice (*O. sativa* L.; Ellis et al., 1993). Seeds of cereals, including barley (Pieta Filho and Ellis, 1991a, 1991b) and wheat (Ellis and Pieta Filho, 1992), increase in seed quality between the end of the seed-filling phase, defined as mass maturity (Ellis and Pieta Filho, 1992) and harvest maturity, when cereal seed crops have dried naturally to about 15% moisture content. Atmospheric [CO_2] affected seed yield per unit area (Wheeler et al., 1996b) and mean seed weight (Wheeler et al., 1996a).

Seeds produced by soybean plants exposed to excessively high temperatures during seed filling are shriveled or abnormal (flattened and wrinkled with depressions in the seed coat), and the quality of these seeds is often much lower than that of seeds with no visible imperfections (Egli et al., 2005). Significant levels of shriveled and abnormal seed would reduce the quality of a seed lot; however, high temperature effects on quality were still evident when shriveled and abnormal seeds were removed and only normal seeds were tested (Spears et al., 1997). Seed quality at harvest (barring mechanical damage) is primarily a function of disease (pod and stem blight caused by *P. longicolla*), temperature, and moisture conditions (Egli et al., 2005). High temperatures during seed filling frequently disrupt normal seed development, which increases the proportion of seeds that are shriveled and abnormal. The quality of these seeds is usually lower, sometimes much lower, than that of normal seeds (Spears et al., 1997). In addition to decreasing seed size and seed composition, drought and/or heat stress can decrease the viability of the harvested seeds. Peanut seeds produced under drought stress had a lower germination rate (Pallas et al., 1977). This was mainly attributed to insufficient calcium level that resulted from impaired calcium uptake under drought conditions (Cox et al., 1976). Similarly, soybean seeds obtained from plants grown at high day (35 °C) and high night (30 °C) temperatures had reduced seed germination and seedling vigor (Gibson and Mullen, 1996). Thomas et al. (2009) showed that the elevated growth temperature during seed production decreases the subsequent percent

emergence and seedling vigor of the seeds and seedling dry matter production of seed produced either at ambient or elevated [CO_2].

Seed vigor is an important parameter that needs to be assessed to supplement germination and viability tests to gain an insight into the performance of a seed lot in the field. Seed quality has a great impact on the quality of planting stock. The uniformity of seed development within the crop is a major factor through which crop production practices and growing conditions will affect seed-to-seed variation. During the growth of field crops, the maximum seed quality is generally regarded to be attained at physiological maturity, that is, at the end of seed filling (Egli, 1998). Seed vigor was often more sensitive to high temperature than was standard germination (Egli et al., 2005). In soybean seed, viability and vigor consistently and progressively decreased as seed size decreased below the mean size. Wetzel's conclusions on the seed size and quality relationships in soybeans have been generalized to most kinds of seeds: The relationship between seed size and quality is most consistent within a lot or population of seeds; the larger seeds within a population are superior in terms of germinability, stand establishment, and survival (Wood et al., 1977).

The crop is harvested at harvest maturity, when seeds have dried to a moisture content that allows harvesting without significant damage. By that time, the seed quality may already have deteriorated. Because seed development within a crop is not uniform, there are differences in the moment when individual seeds reach physiological maturity. In soybean, longer exposure of early pods to deteriorative conditions in the field was believed to explain the lower viability at harvest of seeds from earlier compared with late pods (Illipronti et al., 2000). Poor seed quality can be caused by the effect of pathogens before physiological maturity, preharvest weathering, mechanical damage during and after harvest, and deterioration during storage. Hot, dry weather during seed maturation can also result in poor quality (FAO, 1994). Seed quality is, however, sensitive to temperature during the seed-filling period. Because high temperature can differentially affect the various processes involved in seed filling, warming can affect seed composition. Environmental stresses prevailing during pollen development, germination, and pollen tube growth affect the functioning of the pollen and seed set (Thuzar et al., 2010) and thus the number of seeds and quality. In kidney bean (*P. vulgaris* L.), elevated [CO_2] did not influence seed composition, emergence of seedling vigor of seeds produced at cooler temperatures (Thomas et al., 2009). However, seed produced at high temperature (34/24 °C) had a smaller seed size, decreased glucose concentration,

but significantly increased concentrations of sucrose and raffinose compared with that produced at cooler temperatures (28/18 °C).

A region × year interaction was noted for protein and the sum of protein and oil concentrations (Naeve and Huerd, 2008). Significant year-to-year variability occurs with protein (Hurburgh et al., 1990) and oil (Brumm and Hurburgh, 2006). Although temperature effects on oil concentration may be the best characterized of any environmental factor/quality trait interaction, a definitive model describing this interaction has not been developed. Three groups (Gibson and Mullen, 1996; Pazdernik et al., 1996; Thomas et al., 2003) used average daily temperature ranges of about 22 °C to as much as 39 °C. All the three groups found decreased oil concentrations with higher temperatures (Naeve and Huerd, 2008). Higher temperature increases oil percent and decreases protein percentage. Elevated level of [CO_2], during cultivation of onion and tomato, resulted in an increased photosynthetic rate giving a higher yield as compared to ambient [CO_2]. An increase in temperature during bolting, flowering, and pod setting decreased the seed yield (40% reduction) of cabbage "Golden Acre" in the upper Kullu valley in the Hindu Kush Himalayas from 1962 to 2004 (Agrawal, 2011).

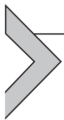
In soybean seeds, elevated temperatures or severe drought during seed development altered tocopherol metabolism, increasing the production of α-tocopherol (Britz and Kremer, 2002). For rice, increasing temperatures caused only slight changes in tocotrenols and tocopherols. However, the proportion of α-tocotrienol and/or α-tocopheral increased at elevated temperatures, whereas γ-tocopherol and γ-tocotrienol decreased (Britz et al., 2007).

6.11. Genetically Engineered Crops

So far, genetically engineered (GE) crops are restricted to a few traits that were developed some 20 years ago: herbicide and insect resistance. No stress-tolerant GE plants have ever been proven to work under real-world conditions. The performance of existing GE varieties under climate stress is so far sobering: However, in recent years, progress has been made by some private companies, such as Monsanto and Pioneer, in maize in drought-tolerant traits. Extreme temperature fluctuations caused crop losses in Bt. cotton in China. Researchers investigating the cause indicated that high temperatures (37 °C) were most probably responsible for a decrease in Bt concentrations in leaves (Chen et al., 2005). Other researchers found that temperature changes, especially a cool period early on in the growing season, caused a reduction in the insect toxicity of Bt. cotton (Cotter and Tirado, 2008).

GE herbicide-resistant soybeans suffered unexpected losses in the United States during very hot spring weather in 1998. Roundup Ready soybeans performed significantly worse than did conventional varieties under conditions of heat stress. The GE soybean stems were more brittle and split more easily, thus allowing infection to occur (Coghlan, 1999). Herbicide-tolerant GE soybeans currently on the market have been reported to have decreased yields of up to 10% compared with traditional varieties (Elmore et al., 2001). Massive use of herbicides in conjunction with these GE plants has already led to an increase in tolerant weed populations (Nandula et al., 2005). For example, 34 cases of glyphosate resistance in nine species have been documented in the United States since 2000 (www.weedscience.org). Now, it is recommended that farmers spray stronger formulas of herbicides (Monsanto, 2008), and mixtures of herbicides (Brooks, 2003), adding to production costs.

Chen et al. (2007), in a multiple generation experiment, compared consumption, growth, and performance of *H. armigera* feeding on transgenic Bt. cotton versus conventional cotton grown under elevated [CO_2] (750 ppm) versus ambient [CO_2] (375 ppm). The results suggest that on the one hand damage caused by the cotton bollworm might be higher under elevated CO_2 conditions, regardless of the cotton variety. On the other hand, population abundance might be lower under elevated [CO_2] as compared to that under ambient [CO_2] (Chen et al., 2007). The researcher explains both observations with nutritional changes under elevated [CO_2] (e.g. compensatory feeding).



7. EFFECT OF CLIMATE CHANGE ON POLLINATION

Climate change is causing changes in the distribution of many plant, insect, and bird species. Pollinators, however, will largely respond by contracting or expanding their ranges according to new climatic patterns. Thus, the possibility of crops losing key pollinating species, or mismatches in the ranges of crops and their pollinators, is a real threat (FAO June 1–5, 2000). Changes in temperature will alter the nature or the timing of species interactions. For example, the timing of plant flowering and the emergence of pollinating insects differ in their responses to increasing temperatures, with potentially large effects on ecosystems and communities (Harrington et al., 1999).

The total economic value of pollination worldwide has recently been estimated at US$ 224 billion, representing 9.5% of the value of world

agricultural food production in 2005 (Gallai et al., 2008). Pollinator-dependent crops—fruits, vegetables, edible oils, nuts, and spices—are generally "high-value" crops. These figures do not include the contribution of pollinators to crop seed production (which can contribute many fold to seed yields), nor to pasture and forage crops.

The Food and Agriculture Organization (FAO) estimates that of the slightly >100 crop species that provide 90% of food supplies for 146 countries, 71 are bee pollinated (mainly by wild bees), and several others are pollinated by thrips, wasps, flies, beetles, moths, and other insects. Additionally, for 75% of food crops (including cereal, legumes, fruits, vegetables, and nuts), a deficit in pollination services reduces fruit quality and/or quantity (Klein et al., 2007). The majority of agriculture is, therefore, potentially affected by pollinator scarcity.

7.1. Pollinator Biodiversity Losses and Disruption of Pollination Timing

Millennium Ecosystem Assessment in 2005 assessed that out of the 24 ecosystem services, 15 are considered to be seriously degraded. Climate change may potentially be one of the most severe threats to pollinator biodiversity (Kerr, 2001). Substantial distribution changes are predicted for groups such as butterflies (Cowley et al., 1999). Another type of loss of synchrony that can occur due to changing climate and have consequences for yield is in insect-pollinated crops, where alterations to the annual temperature cycle can uncouple insect life cycles with crop-flowering phenology (Memmott et al., 2007). Large-scale monoculture, necessary for economic production of mechanically cultivated and harvested crops, provides no continual source of pollen and nectar necessary to maintain strong colonies (Martin and McGregor, 1973). The replacement of natural plant communities by monoculture cultivation is also a factor because most monocultures are not capable of sustaining pollinator populations: wheat and corn do not provide nectar or pollen needs for any bee species (Cane and Tepedino, 2001). The commonly used broad-spectrum insecticides are often as toxic to beneficial insects as they are to the target species (Johanson and Mayer, 1990).

Global biodiversity decline has focused attention on the implications of species losses for the maintenance of ecosystem functioning (Hooper et al., 2005; Tilman et al., 2006). Animal pollination contributes to 35% of global food production (Klein et al., 2007), but anthropogenic activities such as habitat loss, habitat fragmentation, land-use intensification and use

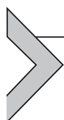
of agrochemicals have adverse effects on pollinator diversity (Biesmeijer et al., 2006; Steffan-Dewenter et al., 2002; Tylianakis et al., 2005), placing crop pollination services at risk (Kremen et al., 2002; Steffan-Dewenter et al., 2005; Tscharntke et al., 2005).

Changing climates may cause changes in the time of growth, flowering, and maturation of crops, with consequent impacts on crop-associated biodiversity, particularly pollinators. Key biological events such as insect emergence and date of onset of flowering need to occur in synchrony for successful pollination interactions. Effective crop pollination is heavily dependent on the biological timing of both the crop and its pollinators. Climate change may have profound impacts on the timing of these events. The honeybee (*Apis mellifera*) is the principal species used for crop pollination worldwide (Free, 1993). Although honeybees pollinate a wide variety of crops, they are often relatively ineffective pollinators on a per-visit basis. Since the 1970s, the number of managed honeybee colonies in the US decreased from >4 to 2.41 million; declines were also reported in Europe. Reductions in bee abundance can cause reduced crop yields (Greenleaf and Kremen, 2006). Non-*Apis* bees, also known as pollen bees, are also valuable for crop pollination (Losey and Vaughan, 2006). Both the abundance and the diversity of wild bee communities are associated with increased crop pollination (Kremen et al., 2004). Honeybees are the principal pollinator used in food production around the world, and they also have declined globally in recent decades (Kevan, 2001; USDA, 2006). Enhancing honeybee pollination efficiency is economically important for hybrid sunflower seed production because fields are frequently underpollinated (Greenleaf and Kremen, 2006).

The timing of pollination is determined by climatic cues such as temperature and water availability (Cleland et al., 2007). Many pollinators also synchronize their life cycles with climatic cues, and this phenological response of plants and pollinators needs to remain broadly synchronized for many plant–pollinator relationships to remain viable. Climate change is altering the phenological response of plants, and some pollinators may be unable to alter their life cycles in synchronization with altered pollination timing. Kudo et al. (2004) show that, since 1998, plants have been flowering much earlier in alpine environments while the time of emergence of pollinators has not necessarily changed, thereby disrupting pollination. Crop pollination shortages are becoming increasingly common so that behavioral interactions between wild and honeybees increase the pollination efficiency of honeybees on hybrid sunflower up to 5-fold, effectively

doubling honeybee pollination services on the average field. Conserving wild habitat at the landscape scale and altering selected farm management techniques could increase hybrid sunflower production (Greenleaf and Kremen, 2006).

Urbanization, deforestation, climate change, nonnative invasive species, unsustainable agricultural practices, and over fishing, among other factors, are modifying the structure of ecosystems and disrupting their proper functioning (Risk governance of pollinator services, 2009). Many crops, through the selective breeding and replication practices of humans, lose their genetic diversity over time. Exposure to pollinators may be one means of introducing a selective influence to maintain genetic diversity. Studies on bottle gourd in Kenya have shown how important a diverse pollinator community is for maintaining the extraordinarily diverse forms of gourds (Morimoto et al., 2004).



8. INTERACTION OF CLIMATE CHANGE FACTORS AND OTHER RESOURCES

Responses of plants to elevated CO_2 often depend on other resources—mainly light, water, and nutrients. Elevated CO_2 increases photosynthesis and yield to an equal extent across all light levels. Despite positive responses, the strongest response would be observed close to the light compensation point. There may be species differences in response to interaction between CO_2 and light. Few studies have shown that shade-loving plants responded more positively to CO_2. It is hypothesized that at low light, plant growth is strongly carbon limited, and thus responds better to high CO_2.

As indicated earlier, elevated CO_2 decreases stomatal conductance leading to decreased water loss, lower total water use, and increased water use efficiency. Thus, under water stress conditions, plants grown at lower water availability will thrive longer on a given amount of water when grown at elevated CO_2. However, these effects will be small due to compensation of water saving from lower transpiration by larger leaf areas and higher tissue temperatures at elevated CO_2.

The response to elevated CO_2 is generally smaller at a lower level of plant nutrients such as nitrogen, phosphorus, or any other or/all nutrients. At low nutrient levels, growth is not limited by carbon availability, because high concentrations of starch and other nonstructural carbohydrates are often observed in plants with low nutrition. Therefore, an increase in the

carbohydrate pool due to increased photosynthesis by elevated CO_2 is not expected to stimulate growth to a larger extent, unless plants grown at elevated CO_2 acquire more nutrients and use them more efficiently. Some studies have shown that nitrogen use efficiency is greater under elevated CO_2 compared with that under ambient CO_2. This is because in most plants photosynthesis is stimulated at elevated CO_2 despite lower leaf nitrogen concentrations.

It is important to understand that although yield responses to elevated CO_2 under resource stress may be relatively higher, the absolute yields will always be smaller, compared to that in nonresource stress environments. In addition to interactions of resources with CO_2, there may be an interaction between these resources themselves. For example, at low water supply, nutrients will be less mobile, and availability and absorption by plants will be lower, and at high light levels, plants will grow faster and may deplete water and nutrients faster.

Elevated UV-B radiation can also influence reproduction and thus seed numbers and seed weight. In general, reproductive organs of most plant species (pollen and ovules) are highly protected by sepals, petals, and ovary walls. In these plants, pollen is susceptible after it falls on the stigma. Exposure to UV-B radiation decreases pollen germination and rate of pollen tube growth by 10–25% in several crop species (e.g. maize, rye (*Secale cerale* L.) and tobacco (*Nicotiana tabacum* L.)). Increased UV-B radiation decreased total pollen production, pollen germination, and tube growth (Koti et al., 2005). Consequently, it affects the fertilization process, which results in fewer seeds in sensitive plants. However, once the pollen tube penetrates the stigma surface, the walls of the style and ovary may provide some protection against UV-B radiation.

Exposure to UV-B radiation caused decreases in the growth of leaves and stems in many plant species in both controlled environment and field studies (Kakani et al., 2003). The decrease in growth of leaves, main stem, and branches is due to reduced cell division rather than to decreased cell size. In regard to exposure to UV-B radiation, the majority of crop species (60%) show a reduction in dry matter production, a moderate 24% show no change, and only 8% of crop species show an increase in dry matter production (Kakani et al., 2003).

The main causes of yield loss are reduced fruit (grain) number due to failure in fertilization, abortion of fruiting structures, and decreased fruit size due to reduced supply of assimilates to the growing sink (fruits). Kakani et al. (2003) reviewed responses of various crops to UV-B radiation in both

controlled environmental and field studies; almost half the studies showed that enhanced UV-B radiation decreased yield, the other half showed no UV-B effect on yield, and a few studies showed increased yields. These differential responses were due to variability in intensity of UV-B radiation. The UV-B radiation in these studies varied and ranged from 2.5 to 63 kJ m^{-2} d^{-1} simulating 10–50% of stratospheric O_3 depletion. In addition, the variable responses can also be due to the differences in the responses of cultivar and crop species to UV-B radiation.

A better understanding of the interaction of elevated CO_2 with other environmental factors associated with global climate change is necessary, as these changes will occur simultaneously. The most important factors other than elevated CO_2 include increases in temperatures, UV-B radiation, and tropospheric ozone levels. The data from several experiments were reviewed and summarized by Poorter and Perez-Soba (2001). These experiments included those on crop species, herbaceous plants, and wild species and tree crops. In summary, there will not be any beneficial effects of elevated CO_2 under nutrient stress, high temperature stress, and higher UV-B radiation. There may be small benefits under low light or drought stress conditions. However, there will be strong beneficial effects with ozone or sulfur dioxide pollution mainly due to partial closure of stomata under elevated CO_2.

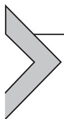
Recent studies on interaction of elevated CO_2 with supraoptimal temperature have shown that the beneficial effects of elevated CO_2 on growth and yield decrease with increasing temperatures (Prasad et al., 2002, 2003). Similarly, total water use will increase under elevated CO_2, and small increases in air temperature would more than offset the water-saving effects of CO_2 due to decreased stomatal conductance. In addition, as indicated earlier, elevated CO_2 increases tissue temperatures, which can be more detrimental to crops. Studies on rice, dry bean, and sorghum have shown that plants grown at elevated CO_2 led to a decrease in optimum and ceiling temperatures for seed sets by about 2 °C (Matsui et al., 1997; Prasad et al., 2002, 2006a). The beneficial effects of elevated CO_2 were also shown to decrease at suboptimal temperatures.

Due to the depletion of the stratospheric ozone layer, higher levels of UV-B radiation is reaching the Earth's atmosphere, which may have a detrimental effect on crops plants. Recent studies on the interaction with CO_2 and UV-B radiation have shown that elevated CO_2 cannot compensate for harmful effects of UV-B radiation. However, studies have also suggested that elevated CO_2 may be able to provide some protection against UV-B

radiation in some plant species. There are a lot of species differences in terms of interaction between elevated CO_2 and UV-B radiation.

There has been a gradual increase in tropospheric ozone, resulting in air pollution. Elevated ozone is known to be deleterious to plants and have a negative impact on photosynthesis, growth, and yield. However, there was a strong positive interaction between elevated CO_2 and increased ozone levels on plant growth and yield. Elevated CO_2 can help to overcome the negative effects of ozone of crops. This is because partial closure of stomata at elevated CO_2 decreases the ozone flux into the leaf, thus decreasing the potential negative effects of ozone on various physiological, growth, and yield processes. However, plant species or cultivars whose stomata are less response to elevated CO_2 may not observe such a positive interaction or will have much less benefits of elevated CO_2 on negative effects of ozone.

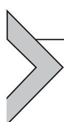
Interaction studies between elevated CO_2 and other environmental pollutants, SO_2 and N_2O, have received far less attention and are not well understood. Studies suggest that these responses could be similar to those of the interaction between CO_2 and ozone; for the same reasons, closure of stomata at elevated CO_2 will lead to a decrease in the influx of these pollutants into the leaf.



9. ABSENCE OF CLIMATE SAFETY NET

The world today is faced with great challenges in being able to produce adequate food, fiber, feed, industrial products, and ecosystem services for the world population. With nearly 80 million people added every year, about 84% of this growth is expected to occur in the developing countries. The increased food supply must come primarily from more intensive cultivation of existing arable land. Added to this stress comes the greatest threat of our life time, the global climate change resulting from increased greenhouse gas concentrations in the atmosphere due to anthropogenic activities (Reddy, 2008). The paradox is that areas that are currently most food insecure will be most affected by climate change. These areas have arguably the greatest need for new crop varieties that are tolerant to extreme climate conditions, especially drought, yet they lack the technology and investment in plant breeding. Support for plant breeding is inadequate in most countries and especially in those countries that need it most (FAO, 2006). In addition, there is also a need for capacity building on programs that support both conventional plant breeders and molecular plant breeding, especially crop physiology and plant biology.

The detrimental effects of climate crisis are not only a matter of geographic vulnerability but they also depend on a region's ability to pay for adaptation measures. For some farmers in Organization for Economic Cooperation and Development (OECD) countries, for example, risks are already mitigated through agricultural subsidies—around US $225 billion in OECD countries in 2005—and through public support for disaster insurance. For poor countries, there is no climate safety net. Even the most basic resources are scarce. Africa currently has one meteorological station for every 25,460 km^2, one-eighth the minimum level recommended by the World Meteorological Organization. By contrast, the Netherlands has one weather station for every 716 km^2 (ETC, 2008a).



10. INTELLECTUAL PROPERTY RIGHTS ISSUES RELATED TO SEED

According to a study by GRAIN (an international nonprofit organization, www.grain.org), most developing countries still mainly depend on farm-saved seed, in particular regions with a large peasant farming sector, such as South Asia and sub-Saharan Africa, where typically 80–90% of planting materials are produced on-farm. In 18 mostly developed countries, typical figures were in the 20–40% range, but for some crops and countries, they are much higher. In several major cereal-producing countries, Argentina, Australia, and Canada, the reported farm-saved seed figures are from 65% up to 95%. Another notable country is Poland; the largest agricultural power in Europe after France, where farmer-saved seed was reported at around 90% for all major crops except oilseed rape (Grain briefing, 2007). In general, intellectual property rights (IPRs) tend to facilitate control over seeds and related knowledge by agribusinesses at the expense of small and subsistence farmers (Cullet, 2003). Maximum productivity growth can come from seeds that constitute a small part (<10%) of the total farm input costs. Most crops, however, contribute the maximum to increased farm productivity. Seed replacement rates in India are lower than in most countries, including China and Vietnam. As a result, yields in India are lower than global averages (Anonymous, 2010a). To take but one example, although the percentage of people engaged in the agricultural sector in the European Union was 20% when the International Union for Protection of New Varieties of Plants (UPOV) Convention was adopted in 1961, the population active in the agricultural sector in developing countries today amounts to 86% of the rural population and 52% of the total population.

10.1. Intellectual Property Rights and Monocultures

Reid (1992) identified a strong connection between IPRs and a bias toward centralized research and believed that this has an impact on agro-biodiversity. He found that the prevailing policy framework for the use of genetic resources for food and agriculture favors 'centralized crop breeding and the creation of uniform environmental conditions, and discourages agro-ecological research or local breeding tailored to local conditions.' IPRs enhance incentives to develop seeds that will have a large potential demand. To ensure the maximum demand for their products, the seed companies tend to focus their research on commonly used high-value crops and develop varieties that can be cultivated as widely as possible. This means either breeding through selection of genes for maximum adaptability or introducing the new seeds while also promoting farming practices that reduce environmental heterogeneity. The biodiversity-erosive effects of this IPR-supported bias toward centralized crop breeding programs are (i) decreased crop diversity; (ii) decreased spatial genetic diversity; (iii) increased temporal genetic diversity due to the need to replace cultivars with new ones every few years; and (iv) increased use of external inputs (WIPO, 2004).

Monopolies in the seed industry lead to the opposite of biodiversity, which is biouniformity. If corporations succeed in obtaining the exclusive property rights to a crop, the rights only apply to a genetically uniform plant variety. This way it contributes to a process in which genetically diverse and traditional varieties are gradually replaced by genetically uniform modern seeds. Highly concentrated seed markets lead to even less genetic variety and fewer new breeds. For example, on the US seed market, farmers saw the gradual substitution of conventional seeds (e.g. soybean and cotton) with genetically modified (GM) seed. Conventional seeds became short in supply for the simple reason that they are less profitable for the seed industry. This development toward biouniformity is a threat to food security because genetic uniformity makes crops vulnerable to diseases and pests (A Seed Europe, 2010).

The use of high-input selection environments in a market-driven agriculture has been largely responsible for the trend of modern plant breeding toward narrowing the genetic base of our crops accompanied by a trend toward homogeneity: one clone, one pure line, one hybrid (Simmonds, 1983). Uniformity and broad adaptation are very useful attributes to accommodate large-scale centralized seed production (Davis, 1990). Although the merits of genetic uniformity have been questioned in developed countries (Wolfe, 1991), it is still very popular in breeding programs and seed

production systems of developing countries at both the national and international levels. This is in contrast with the genetic diversity that characterizes agriculture in marginal environments. The IPRs alone cannot be held responsible for the loss of agro-biodiversity, but IPRs are bound to encourage the displacement of a wide diversity of traditional local varieties in favor of a small number of widely adapted hybrids and homogeneous modern varieties. Moreover, one of the lessons of the Green Revolution is that the development of new varieties by the seed industry is unlikely to match the loss of traditional varieties after these new varieties are introduced (Kothari and Anuradha, 1997).

10.2. Monopolization by Transnational Corporations

The commercial seed industry has undergone tremendous consolidation in the last 40 years as transnational corporations entered this agricultural sector and acquired or merged with competing firms. This trend is associated with impacts that constrain the opportunities for renewable agriculture, such as reductions in seed lines and declining prevalence of seed saving (Howard, 2009). Multinational pharmaceutical firms transformed themselves into 'life science' conglomerates, and divested commodity chemical divisions, in anticipation of synergies between newly integrated agricultural biotechnology activities and their pharmaceutical divisions. The life science firms formulated strategies involving sales of crop protection traits to seed producers via arms-length IPR transactions. Agricultural biotechnology startups proliferated in the 1980s and 1990s in the United States, financed by venture capitalists, often built around patented innovations produced by university scientists, government agencies, and licensed exclusively to the startups (Wright and Pardey, 2006). The integration of most US-American and European large seed companies with modern biotechnology companies during the late 1990s was not incidental. Having access to plant material is a necessary ingredient for developing transgenic plants. To make money with these plants, large-scale production sites and distribution networks are necessary. Biotechnology and the plant breeders/seed producers turned out to be a perfect match, acting jointly to promote genetically modified organisms (GMOs) in agriculture. After Monsanto's successful takeover of the US soybean seed market in 2007, the majority (>90%) of all soybean plantations in the United States were carrying transgenic traits by Monsanto. This illustrates well that the predominance of GE varieties equals with market dominance of multinational biotech corporations (A Seed Europe, 2010).

Although concern that monopolization has proceeded too far is belatedly spreading (Brennan et al., 2005), in economic terms, the transgenic technologies have generated large economic value, the majority of which seems to go to farmers or consumers. The leading agricultural biotechnology firms at present see insufficient prospective returns in most other agricultural or horticultural crops, whether in developed countries where such crops have been called 'orphans' or in developing countries. Concentration in the global agricultural biotechnology industry is inevitable, given the high fixed cost and low variable costs associated with the technology (Wright and Pardey, 2006). Over the last two decades, there has been a significant concentration in the commercial seed industry mainly in industrialized countries. According to estimates of the International Seed Federation, in 1985, the 10 largest seed companies accounted for approximately 12% of the market, increasing to almost 40% in 2007. After decades of seed industry mergers and acquisitions, accompanied by a steady decline in public sector plant breeding, the top 10 seed companies control 57% of the global seed market (ETC group, 2008a). In another publication by the same group, the top 10 seed companies account for US $14,785 million or two-thirds (67%) of the global proprietary seed market (ETC group, 2008b).

The theories of eroding obstacles to accumulation in agriculture sector include dynamic mills that enroll farmers in this process, and consolidation to reduce competitive threats. These theories help to explain why seed industry consolidation is rapidly expanding in new directions—horizontally, vertically, and globally. The result is increasing monopoly/oligopoly power for a decreasing number of transnational corporations. This concentration of power is fundamentally incompatible with renewable agricultural practices that are barriers to large-scale capital accumulation, such as saving and replanting seed (Howard, 2009).

10.3. Seed Price of Conventional Versus GE Seeds

In developed countries, the seed cost ranged from 5 to 15% of the total operating costs until 1995, when they started to increase for corn, soybean, and cotton, whereas wheat seed costs declined. A sharp increase in the contribution of seed costs to the total operating costs occurred in the last five years for corn, soybean, and cotton, which is when the wide use of transgenic cultivars occurred for these crops (TeKrony, 2006). The seed price at the international level is fixed according to different components of seed technology such as, genetic (60%), chemical treatment (7–8%), disinfection

(2–3%), pelleting/coating (12–12%), priming (10%), calibration, and physical impairment (8–10%) (Le Buance, 2008).

The USDA estimates that seed expenses for farmers increased by 66.3% from $10.4 billion in 2005 to $17.3 billion in 2009, more than double the increase in the total farm production expenses (Clapp, 2009). DuPont Pioneer Hi-bred announced a 20% increase for corn seed and 35% increase in soybean seed in 2009 and is projecting "double-digit seed price increases" between 2009 and 2013 (Food and Water Watch, 2009). One other consequence of monopolization in the seed industry is the increases in seed prices, the companies manage to force on farmers. In soybeans, farmers have traditionally paid about a two-fold premium for purchased soybean seed, as compared to the grain price of soybeans. The roundup ready, RR2 soybean seed-to-soybean price ratio will be around 7.8, over three times the historic normal. For conventional farmers from 1975 to 2000, soybean seed prices rose a modest 63%. Over the next ten years, as GE soybeans came to dominate the market, the price increased an additional 230%. The $70 per bag price set for RR 2 soybeans in 2010 is twice the cost of conventional seed and reflects a 143% increase in the price of GE seed since 2001. These observations are similar in conventional and GE maize seed. In 2009, the GE corn-to conventional grain corn seed premium was 69%, with GE seeds costing $235 per unit. Conventional corn seed prices were <$100 per unit through 2007. Corn growers planting the first-ever, eight-traits stacked GE variety called "SmartStax" corn will pay about 2 times more per unit than do farmers planting conventional seeds, and almost four times more than conventional farmers do just ten years earlier. GE cotton seed price inflation has dwarfed the pace of increases in the price of GE soybean and corn. From 1975 to 1996, the price of cotton seed only doubled, but in the GE cotton era, it has risen from $73 to $589 per Centum weight (CWT). Today, GE cotton seed costs $700 per CWT, an amazing 5.9-fold more than conventional cotton seed do (A Seed Europe, 2010; Benbrook, 2009).

10.4. Yield of GM Crops

In the US, improved yields cannot be solely attributed to biotechnology because corn and soybean yields have not increased faster since GM seeds were introduced. The average corn yields increased by 2.7% between 1995 and 2009, matching the 2.7% yield increase observed between 1981 and 1995, before GM seeds were available (USDA, 2009). Soybean yields increased faster between 1981 and 1995 (2.1% annually) before GM, but yields rose by only 1.6% between 1995 and 2009. Herbicide-tolerant corn

and soybeans had no yield increase over non-GM crops but found a slight yield advantage (3–4%) for insect-resistant corn. The USDA survey of yield studies found that herbicide-tolerant biotech corn seeds did not increase yields for farmers. Barney (2007) observed that conventional fields produced 10% more than biotech soybean fields did, while Elmore et al. (2001) reported that conventional lines had 5% higher yields than did GM herbicide-resistant sister soybean lines; high yield conventional soybean lines had 10% higher yields than the GM fields did. A University of Saskatchewan study found that conventional canola had 8.2% higher yields than did Roundup Ready canola (Food and Water Watch, 2009). With regard to transgenic crops in developing countries, however, the Bt. cotton revolutionized cotton production in India since its introduction in 2002. Farmers have increased planting of Bt. cotton from 29,000 ha in 2002–2003 to 10 million ha in 2010. The average yield in cotton has increased from 300 kg ha^{-1} in 2001–2002 to 560 kg ha^{-1} in this period. In 2007–2008, cultivation of Bt. cotton in India has resulted in a 31% increase in yield, 39% reduction in pesticide usage, and >80% increase in profitability for farmers (Rana, 2011).

10.5. Environmental Issues Related to GM Crops

A dozen years after GM crops were introduced commercially; weeds and insects have already become resistant to the genetic traits that are intended to prevent infestations. This reduces farm yields and/or income and encourages farmers to apply more and more potent herbicides and pesticides. At least eight weed species in the United States (and fifteen worldwide) have been confirmed to be resistant to glyphosate (Clapp, 2009). Wenzel (2007) reported that corn rootworms could pass on Bt. resistance to their offspring that are considerably more resistant to the GM crop than the offspring of corn rootworm beetles that had not been exposed to Bt crops. However, total herbicide use can increase with herbicide-tolerant crops. Farmers increase application of the herbicide tied to the crop trait even though they may reduce the application of other herbicides. About 62.5 million-kg increase in herbicide applications overwhelmed the 7.2 million-kg decline in insecticide applications attributable to Bt. corn and cotton. Since 1994, glyphosate use in soybeans, corn, and cotton increased 15-fold to about 54 million-kg in 2005. Between 2002 and 2006, in addition to glyphosate applications, the amount of the herbicide 2,4-D applied to soybeans doubled to combat weeds. This increased agrochemical use can result in run-off from farm fields, pollute water, and degrade soil (Food and Water Watch,

2009). However, Brookes and Barfoot (2009) reported that in 2007 alone, a year when GM crops were grown on only 7% of arable land in the world, the total reduction due to both the direct and indirect emission effects of GM crops amounted to over 14,200 million kg of CO_2, the equivalent of removing >6 million cars from circulation.

10.6. Net Farm Income Using GM Crops

From 1975 to 1997, soybean farmers spent 4–8% of crop income on purchased seed. In 2009, farmers planting GE soybean seeds spent 16.4% of soybean cash market income per acre on seed—twice the historic norm. It was predicted that farmers planting RR 2 soybeans in 2010 would commit 22.5% of gross income per acre to the purchase of these GE seeds. Corn growers spent 4–11% of gross market income per acre on seed from 1975 through the beginning of the GE era in 1996, and 11–17% of operating costs per acre. GE corn seed, however, has become much more expensive as a percent of gross income and operating costs. In 2009, GE corn seed accounted for 19 and 34% of gross income and operating costs per acre, about twice the historic norms. The cost of GE cotton seed has helped drive net farm income on cotton farms into the negative since 2008. In the GE era, the average net returns on cotton farms have dropped by roughly $200 per acre, and the cost of GE cotton seed has increased almost $100 per acre. Obviously, many factors have contributed to the declining profitability of cotton production, but two of the most important are increase in seed costs and the need to apply more herbicides in an effort to control glyphosate-resistant weeds, particularly in the Southeast (Benbrook, 2009). However, in India, the profitability of farmers increased using transgenic cotton to the tune of >80%, which could be among various factors due to seed price control mechanisms imposed by State governments.

10.7. IPRs and Technology Use Agreements Related Issues

Since the early 1990s, corn, soybean, and cotton breeders in the US have focused predominantly on the incorporation of proprietary pest-management-related traits into elite germplasm, using the tools of biotechnology. GE seeds now account for the vast majority of the new seed varieties offered for sale by the major seed companies. Other important goals traditionally pursued by plant breeders have taken a back seat. The seed price and farm income consequences of this historic shift in control over plant breeding from the public sector, and goals advancing public welfare, to the private sector and its basic goal of maximizing return to shareholders' equity

through expanding market share and profit margins (Benbrook, 2009). A brief description of biotech-stacked traits and stewardship through technology use agreement is given in the following sections.

10.8. Biotech-Stacked Traits and Patent Infringements

The United States is the global leader in GM production, with half the world's cultivated acres in 2008 (ISAAA, 2008). Presently, 25 countries allow the commercial production of GM crops, with the five largest producers accounting for 91% of GM cultivation. In the United States, biotech seeds are grown on nearly nine out of ten acres of corn, soybeans, and cotton— 85% of corn, 91% of soybeans, and 88% of cotton (Food and Water Watch, 2009). In 2009, 25 countries planted 134 million ha with biotech crops, which is a significant increase of 7% (or 9 million ha) over 2008. The projections for 2015 with regard to adoption of biotech crops in 40 countries are 20 million farmers planting biotech crops and area seeded with these crops is projected to be 200 million ha (Anonymous, 2010b).

Monsanto introduced its first double-stack trait variety in 1998, and its first triple-stack trait in 2005. Syngenta aims to make triple-stack maize account for 85% of its portfolio. Whether farmers want the premium-priced, fully loaded stacked traits or not, they may have little choice. Monsanto and Dow Agrosciences joined forces in 2007 to develop maize seeds with up to eight genetic traits (two kinds of herbicide tolerance and six genes for insect resistance) (ETC group, 2008b). The product was commercialized in 2010. Giant transnational corporations such as Monsanto, BASF, DuPont, Syngenta, Bayer, and Dow (and their biotech partners) have filed 532 patent documents on the so-called "climate ready" genes at patent offices around the world. The 532 documents represent 55 patent families (corresponding to a single "invention" submitted for patent monopoly in more than one country). Together, six of the world's largest agrochemical and seed corporations (BASF, Monsanto, Bayer, Syngenta, DuPont, and Dow) account for 42 of the 55 patent families (79%). These six companies collectively control around half of the proprietary seed market and 75% percent of the global agrochemical market (ETC group, 2008b). The top three seed firms currently control 85% of transgenic corn patents, and 70% of nontransgenic corn plant patents in the United States (Glenna and Cahoy, 2009). Although the Global South is the nucleus of the majority of the world's agricultural biodiversity, the seed industry is dominated by firms from North America and Europe that use intellectual property protections to exploit this wealth (Eaton and Louwaars, 2009).

10.8.1. Stewardship

The owner of an invention has a vested interest in its stewardship. Without the cooperation of the farmer–customer, the value of the invention may be significantly reduced or even lost. Therefore, Technology Use Agreements and associated stewardship and product guides, when applicable, try to ensure that appropriate guidelines are known to the farmer–customer, and that the farmer–customer will adhere to them. Obvious examples include refuge requirements to prevent the development of tolerance in the insect population, any pesticide restrictions (same) and grain channeling restrictions to keep GMO grain in countries where the trait is approved. Following guidelines of application rates, timing and rotations are critical components of stewardship. These stewardship provisions make good sense for preserving the usefulness of the trait, and/or are required by federal/national, state/province, or local authorities (UPOV, 2008).

10.8.2. Patent Infringement

The Monsanto, the world's largest seed corporation, through its technology use guide system, mails an updated agreement to farmers each year, and farmers that continue to use Monsanto's technology after receipt of any new terms are automatically agreeing to be bound by the new terms. Farmers who discontinue their use of Monsanto's GE seed face patent infringement allegations in the event that some of that seed from the previous year sprouts "volunteers" in fields converted to conventional varieties. By growing these volunteers and harvesting them along with the rest of their crop, farmers could be considered to be "using" Monsanto technology, despite not having purchased Monsanto seed that year. This inadvertent use, in combination with receiving a new technology agreement from Monsanto, could constitute tacit acceptance of any new terms outlined in the agreement, thereby exposing the farmer to patent infringement prosecution. The Technology Agreement exposes the seed-purchasing farmer to a huge financial liability. Should the farmer ultimately be found legally responsible for breaching the terms of the technology agreement, bankruptcy is not an uncommon outcome. The agreement provides that if a grower saves, uses, sells, or acquires seed for planting in violation of the agreement, the grower is liable to Monsanto for patent infringement. This liability can also lead to the grower paying Monsanto and its licensed technology provider(s) for their attorneys' fees and costs of enforcing the agreement (CSF, 2005. Monsanto versus US Farmers. www.centerforfoodsafety.org).

Farmers pay a licensing fee to use patented seeds and sign a contract with the biotechnology company that gives the farmer limited permission to plant the patented seeds for a single crop season. By October 2007, Monsanto had filed 112 lawsuits against farmers for patent infringement, recovering between $85.7 and $160.6 million from farmers in court rulings and out-of-court settlements. It is a well-documented fact that a farmer's field could be inadvertently contaminated with GM material through crosspollination and seed dispersal, and at least one farmer contends he was sued when his fields were inadvertently contaminated with GM crops from neighboring farms (Food and Water Watch, 2009).

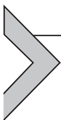
The global seed giants, Monsanto, DuPont, and Dow AgroSciences (all three from the United States), Bayer Crop Science and BASF (both from Germany), and Syngenta (Switzerland) controlled >50% of the global proprietary seed market and at the same time 75% of the global agrochemical market in 2007. With regard to seed sale, all three US companies (Monsanto, DuPont, and Dow AgroSciences), Syngenta, and BASF use the technology use agreement, while Bayer Crop Science does not use it.

10.9. Biosafety Issues Using Transgenic or Genetically Modified Crops

To achieve any significant progress in agriculture, the availability of high-quality seed of the improved varieties at a reasonable price is a prerequisite. Significant changes in plant breeding, seed multiplication and trade have been brought about by modern agricultural practices combined with the establishment of the World Trade Organization and Trade-Related Aspects of Intellectual Property Rights, including Plant Breeders' Rights. As more and more seed is being moved around the globe, regulations have been put in place to guarantee a sustainable supply of high-quality seed. As a result, the industry today is faced with more and more regulations, particularly in intellectual property and variety registration, seed certification, and phytosanitary matters. Recent developments show an increase in regulations in relatively new sectors such as organic seeds, biotech varieties and chemically treated seeds (Bruins, 2009).

Biosafety liability issues typically arise in situations where non-GM seed, crops, or food is adulterated by the unintended presence of GMOs. This liability may arise in relation to Consultative Group of International Agricultural Research (CGIAR) Centers, National Agricultural Research Centers, GM seed manufacturers/suppliers, farmers (GM, non-GM, organic-,

and GM-free), contract harvesters, bulk handlers, local transporters, produce manufacturers, and retailers.



11. CONCLUSIONS

There are wide geographical variations in the expected climatic conditions over the twenty-first century. The variability of crop yields has increased since the beginning of the century as a consequence of extreme climatic events, such as the drought and summer heat of 2003 and the spring drought of 2007 (SEC, 2009). A cryptic yet critical threat to biodiversity is the loss of future evolutionary potential. Extinction of genetically distinct populations, decreases in effective population sizes, and homogenization of habitat types are all likely to have negative effects on future biodiversity (Myers and Knoll, 2001; Woodruff, 2001). The abundance and diversity of wild bees as well as the abundance of honeybees are now declining, and some species are clearly at risk (Stokstad, 2007).

In many developing countries, however, farmers' access to quality seed of a diverse range of adapted varieties has been impeded by factors such as weak seed production and distribution systems, poor seed quality assurance systems, lack of information about adaptation and quality of seed available in the market, lack of access to and credit for inputs, and inadequate seed policies. Further, the pressure from increasing food prices and climate change creates additional challenges. It has exposed the vulnerability of food-insecure households that are net food buyers and who often rely on food grain for seed. The prices of seed and other agricultural inputs have increased, leading to the lack of access and stagnation in production in developing countries (FAO, April 22–25, 2009).

Under climate change, pests associated with specific crops may become more active (Coakley et al., 1999; IPCC, 1996). Increased use of agricultural chemicals might become necessary, with consequent health, ecological, and economic costs (Chen and McCarl, 2001; Rosenzweig et al., 2002a, 2002b). Costs of production are likely to rise in a changing climate, as producers adjust crop varieties and species, scheduling of operations, and land and water management (Rosenzweig et al., 2001). Reynolds et al. (2001) observed that continual heat stress affects approximately one million ha of wheat in developing countries, while terminal heat stress is a problem in 40% of the temperate environment, which covers 36 million ha. Similarly, the cost of seed production will also be high in two ways: first to control

insect pests, disease and weed management, and second, in seed processing because due to terminal heat and/or drought stresses the ratio of small/abnormal/shriveled seed is expected to be high, thus resulting in low ratio of normal sized seed. Therefore, the actual percentage of acceptable seed would be less and would increase the cost of seed indirectly.

Agricultural biotechnology has revolutionized plant breeding in ways that relax some of these agronomic constraints. For example, breeders can now transfer and insert new crop traits with precision. These techniques can streamline the process of adapting varieties for local conditions, but they can also raise a new set of potential impediments in the form of biotechnology regulations and IP constraints. A lack of biosafety regulations in developing countries, in addition to restrictions on GM crops emanating from developed countries, have made it difficult for developing countries to take advantage of new downstream technologies (Lybbert and Sumner, 2010).

Although it is necessary to focus on seed systems, it is also imperative to realize that seed constitutes only one of the many inputs necessary for rural livelihoods (Longley et al., 2001). As seed multiplication is an essential part of crop production, attending to production constraints will help increase farm families' access to own-saved seed more sustainably. Local markets are an important source of off-farm seed. Hence, a well-developed seed marketing system also helps increase access. Both the formal and informal seed systems need better inputs, including funds for research, and awareness and implementation of agricultural technologies. Future agricultural development is predicated on the ability of a reliable and sustainable commercial seed industry to supply enough quality seed and complementary inputs to small farmers to boost agricultural productivity and produce marketable surpluses (ICRISAT, 2010). Overall, there are several challenges in production of high-quality seed and increasing agricultural productivity in the current climate to meet the increasing population; these will increase with changing climates. There is a need for investment in research, education, capacity building in the area of seed production, plant breeding, agriculture, and postharvest storage pest management.

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CHAPTER THREE

Elucidating Mechanisms of Competitive Sorption at the Mineral/Water Interface

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Contents

Abstract

This chapter provides insights into the current state of knowledge on the factors that influence the competitive sorption of different ions on soil components and soils. In soil environments, different cations, anions, and molecules are present together, and then mutual interactions occur among them for sorption on soil components (phyllosilicates, humic substances, variable charge minerals, carbonate, and microorganisms), organo-mineral complexes, and soils. The influence of competitive sorption on ion partitioning between two ions received great attention, but studies on the relative competition for sorption sites onto soil components or soils among various ions are rather scant. In the past decades, many researchers have carried out molecular-scale studies

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to couple macroscopic sorption investigations with direct characterization by spectroscopic techniques. A wide array of models has been also used to describe chemical species structures and distribution in solution, at the solid–solution interfaces, and in the solid phase. This chapter first gives information on the factors that affect the sorption of cations and anions on phyllosilicates, organic matter, variable charge minerals (mainly Al- and Fe-oxides), and microorganisms, when added alone, and their capacity to form *outer*- or *inner*-sphere complexes on the surfaces of the soil components. Then, attention is given to the influence of the nature and concentration of inorganic and organic anions on the sorption of metal cations. The capacity of inorganic and organic ligands to enhance or inhibit cation sorption is discussed. Sorption increase is due to the formation of type A (metal ion bonded to both the sorbent and the ligand) or type B complexes (in which the ligand is bonded to the sorbent between the surface and the metal) on the surfaces of the sorbents or the formation of surfaces precipitates or by electrostatic interactions, whereas sorption decrease of a cation may occur when a ligand blocks common surface sites. The competition of two or more metal cations and the effect of an anion on their competition in sorption have also been discussed. The sorption of an anion inhibits the sorption of the competing anion(s) because it reduces the number of sites available on the sorbent (mainly variable charge minerals) and through changes in the electric potential. The competition in sorption between two or more ligands is affected by many factors such as pH, the nature of the sorbent, the affinity of each anion for the surfaces of the sorbent, the relative concentration of each anion, the surface coverage of the ligands, the sequence of addition of the competing anions, and the residence time. Particular attention has been given to the competitive sorption involving sulfate and phosphate as affected by other inorganic and organic ligands. Competitive sorption involving other ligands (e.g. carbonate, silicate, arsenate, arsenite, selenite, selenate, chromate, humic (HA) and fulvic acids (FAs), and selected low-molecular mass organic ligands (LMMOLs) has been also considered.

In memory of my beloved wife Raffaela (Lella)
Sweet friend, precious confident and wise advisor
A special person I had the great privilege to have always on my side

1. INTRODUCTION

Soils have the ability to replace ions from soil solution by sorption reactions.

Dynamics and mobility of cations, anions, and uncharged species in soil environments are significantly influenced by sorption/desorption reactions. However, sorption involves not only *adsorption* to the surface of a particle but also implies migration of *adsorbed* sorbate into the interior of the particles, coprecipitation, surface precipitation, precipitation, and mineral transformations (Ford, 2012). Here, the term sorption will be used to indicate

the uptake of soluble sorbates without relation to a specific retention mechanism. In this way, no mechanism (adsorption, partitioning, precipitation, and diffusion) is implied. Because it is very difficult to distinguish between so many processes, the term sorption is used to indicate that the exact retention mechanism is unknown.

All soil components are able to sorb cations, anions, and uncharged species, but they greatly differ in their sorption capacities and binding energies. In fact, even a single soil component (e.g. a phyllosilicate or a metal oxide), typically has different kinds of sorption sites that span a range of binding energies (Jackson, 1998).

When an ion or molecule, present in soil solution, reacts with a surface functional group of a soil component, a surface complex is formed. There are two types of surfaces complexes, *inner-sphere* and *outer-sphere*. An *inner-sphere* surface complex has no water molecules interposed between the surface functional group and the small ion or molecule it binds, whereas an *outer-sphere* surface complex has at least one interposed water molecule (Sposito, 2000). Ions or molecules forming *inner-sphere* complexes on the surfaces of a sorbent are said to be specifically sorbed, indicating that they are strongly held through bonds with a covalent character. Vice versa, ions retained by *outer-sphere* complexes are said to be nonspecifically sorbed. Specific sorption is not completely understood, because it may involve different mechanisms. For example, sorbed metal species may range from simple cations to polynuclear hydroxides of different compositions forming by hydrolysis in solution or by nucleation on the surfaces of a colloid.

A variety of spectroscopic techniques are used to study the structures of ion surface complexes in situ at mineral–water interfaces. Many researchers have carried out molecular-scale studies to couple their macroscopic sorption studies with direct in situ characterization by different spectroscopic techniques, which include, inter alias, Raman and Fourier transform infrared (FTIR) spectroscopy, magnetic resonance spectroscopies including electron spin and paramagnetic resonance (ESR or EPR), X-ray absorption spectroscopy (XAS), extended X-ray absorption fine structure (EXAFS) spectroscopy, X-ray absorption near edge structure (XANES) spectroscopy (Bertsch and Hunter, 1998; Borda and Sparks, 2008; O'Day, 1999; Manceau et al., 2000; Sparks, 2003; and references therein). The binding of metal cations to humic substances has been reviewed in detail by Senesi and Loffredo (2005, 2008). A wide array of models are also available and used to describe chemical species structures and distribution in solution, at the solid–solution interfaces, and in the solid phase (Goldberg and Criscenti, 2008).

Cations and anions sorbed on the surfaces of clay minerals, and other soil components may show different types of surface complexes and molecular configurations (Ford, 2012; Sparks, 2003, 2005). A huge amount of literature exists on the sorption of single metal cations and anions onto soil components, based on both macroscopic and molecular-scale investigations. Many factors such as pH, surface coverage, ionic strength, nature, physicochemical properties of the surfaces of the sorbent, nature and oxidation state of the sorbate, and residence time affect the type of the complex formed by a single metal cation or anion. For example, Goldberg and Johnston (2001) demonstrated that arsenate form *inner-sphere* surface complexes on both noncrystalline Fe- and Al-oxide, whereas arsenite forms *inner*- and *outer-sphere* surface complexes on Fe-oxides and *outer-sphere* surface complexes on Al-oxides. By using XANES spectroscopy, Ona-Nguema et al. (2005) showed that arsenite forms similar surface complexes on ferrihydrite and hematite that differed from those formed on goethite and lepidocrocite. Further, the dominant complex type also differed from mineral to mineral with the surface coverage. Recently, it has been ascertained that variations in hematite particle sizes (nanoparticles and microparticles with the average diameters of 10.5 and 550 nm, respectively) affect the modes of Zn sorption, leading to the formation of different types of surface complexes and/or precipitates (Ha et al., 2009a, 2009b). The heterogeneous nature of the soil constituents, usually associated with each other to form complexes, adds further complexity to the interaction of metal cations, anions, and neutral substances onto the soil.

However, in soil, mutual interactions occur among different ions and molecules, competing with each other for sorption sites of the sorbents. Interactions between different anions or cations for sorption on soil components are of paramount importance in understanding chemical processes in soil environments. The fundamental understanding of the sorption processes that control cation and anion mobility and bioavailability in soil requires a knowledge of the factors that affect their interactions with other ions and substances present in soil solution (Ostergren et al., 2000a).

During 1992–2012, macroscopic and spectroscopic studies, dealing with competition in sorption between two or more ions on soil constituents and soils, significantly increased.

The mutual influence of different ions in competing for sorption sites of a sorbent is caused by site competition and/or electrostatic effects. As will be discussed below, usually, for ions with the same charge, these two effects have a negative mutual influence on the sorption, whereas for ions with

the opposite charge, the electrostatic effect has a positive mutual influence on the sorption; however, for competitive sorption of cations and anions, a combination exists between synergy and competition.

This chapter aims to provide the current state of knowledge on the factors that affect the competition in sorption of cations and anions, both nutrients and pollutants, on soil components and soils. The chapter first discusses the processes that control the sorption of cations or anions added alone as well as the types of the complexes and molecular configurations they may form on clay minerals and organic matter. Then, special attention is given to the effect of organic and inorganic ligands on the sorption of cations, the competition in sorption between two or more cations, and, finally, the competition in sorption between two or more anions.



2. SORPTION OF CATIONS AND ANIONS ONTO SOIL COMPONENTS

The soil components responsible for cation and anion (nutrients and pollutants) sorption include phyllosilicates, soil organic matter, carbonates, variable charge minerals (crystalline and short-range ordered Fe-, Al-, Mn-oxides, phyllosilicates coated by OH–Al and OH–Fe species, allophanes, imogolite), and microorganisms. Soil components differ greatly in their sorption capacities, their cation and anion exchange capacities, and the binding energies of their sorption sites. Soil colloids are usually present together and not as separate entities. The mutual interactions between phyllosilicates, metal oxides, humic acids (HAs), and/or microorganisms promote the formation of complexes with peculiar sorption properties and significantly affect the sorption of nutrients and pollutants. Many studies have been carried out on the sorption of cations and anions onto organo-mineral complexes (Huang, 2004; Violante and Gianfreda, 2000; Yuan and Theng, 2012).

As reported before, two general surface complexes exist and are described by the configuration geometry of the sorbate at the sorbent surface. These include *inner-* and *outer-sphere* surface complexes (Borda and Sparks, 2008; Sparks, 2003; Sposito, 1986). *Outer-sphere* complexes involve electrostatic coulombic interactions, and are thus weak compared with *inner-sphere* and are reversible. *Inner-* and *outer-sphere* complexations can occur simultaneously (Catalano et al., 2008; Sparks, 2003). However, where more than one type of surface species is present, XAS bulk will detect only the primary (or average) type of surface product/species in the bulk sample (i.e. sums over

all geometric configurations of the target atom). Consequently, although it may be concluded that the primary surface complex is *inner-sphere*, this does not mean that *outer-sphere* complexation is not occurring. Recently, by using X-ray scattering measurements to study metal(loid) binding on single crystal surfaces, Catalano et al. (2008) showed that arsenate surface complexation was bimodal, with sorption occurring simultaneously as *inner*- and *outer-sphere* species.

The strength of the sorption of cations and anions is an important factor governing their mobility in soils.

The first step of sorption of ions on the surfaces of minerals and soils is sorption on external surfaces, followed by solid-state diffusion into the particles and sorption on the internal surfaces. The diffusion processes are very slow and may continue for months (metal oxides) or years (soils). Fischer et al. (2007) studied the sorption of many cations (Pb, Hg, Cd, Zn, Cu, Ni, Co, Mn, Cr, and Al) onto a goethite from 2 h to 8 weeks. The metals differed in the rate to continue to react with the surfaces of goethite, the rate of reaction being correlated with the ionic radius of the metals; the smaller the radius, the faster the reaction.

2.1. Sorption of Cations

The sorption–desorption processes of cations differ greatly from those of anions. Cations show typical ion exchange behavior on phyllosilicates. Surface bonding is prevalently electrostatic, and the high selectivity of some phyllosilicates for heavy metals (relative to metals as Ca or Mg) may indicate the presence of –SiOH or –AlOH groups capable of chemisorbing these metals. The uptake mechanisms of cations by phyllosilicates depends on pH, metal concentration, ionic strength, and reaction time. Angove et al. (1997) demonstrated that at low pH, Cd sorbs to the faces of the kaolinite crystals, which carry permanent negative charge, but >pH 5.0, it sorbs on variable charge edge sites, which are similar to those of (hydr)oxides. Sorbed metal cations on the edge sites and planar sites of montmorillonite have been identified by EXAFS as *inner-sphere* and *outer-sphere* complexes, respectively (Sparks, 2003, 2005).

Organic matter and metal oxides are much more effective scavengers of polyvalent cations, because specific sorption and other complexation processes may be the dominant binding mechanisms (Huang and Germida, 2002; Violante et al., 2008b). The natural organic matter ranges from high molecular weight humic substances to simple multifunctional organic molecules and is present in soil–water systems as dissolved species, colloidal

material, and coatings on Al- and Fe-(hydr)oxides (Senesi, 1992; Senesi and Loffredo, 2005; Sparks, 2003; Violante and Gianfreda, 2000). Humic substances behave as a natural "multiligand" complexing system (Senesi and Loffredo, 2005, 2008). They contain a variety of functional groups such as carboxyls (–COOH), phenols (–OH), thiols (–SH), and amines ($-NH_2$) and show a strong binding ability for polyvalent cations (Ca, Mg, Al, Fe) and trace metals. The high degree of selectivity of soil organic matter for polyvalent cations (e.g. heavy metals) indicates that they form *inner-sphere* complexes with the functional groups, often forming internal five- or six-member ring on structures (Huang and Germida, 2002; Karlsson et al., 2006; Manceau and Matynia, 2010; Senesi, 1992; Sparks, 2003). Senesi and Loffredo (2008) have described the spectroscopic techniques for studying metal–humic complexes in soil.

The ability of variable charge minerals to retain metal cations is strongly pH dependent. Spectroscopic techniques, such as ESR and EXAFS, have been used for the identification of metal complexes at the surfaces of Al-, Fe-, or Mn-oxides, and silicate clays (Sparks, 1999, 2005, 2008). ESR spectra of Cu- on Al-hydroxide and allophane showed that this metal was coordinated directly to surface Al–OH (or Si–OH) groups, forming monodentate and bidentate complexes (McBride, 1989). The mechanism of most polyvalent cation association with hydrous oxide surfaces involves an ion exchange process in which the sorbed cations replace bound protons. Usually, specifically sorbed cations raise the value of the point of zero charge (PZC) of variable charge minerals. pH affects the sorption of metal cations, either by changing the number of sites available for sorption (sorption increases by increasing pH) or by changing the concentration of cation species (Me^{2+}, $MeOH^{+}$, $Me(OH)_2$).

As reported by Violante et al. (2008b and references therein), the following affinity series for freshly precipitated Fe-, Al-, and Mn-oxides has been recorded for selected polyvalent cations:

$$\text{Fe-oxide: Pb} > \text{Cu} > \text{Zn} > \text{Ni} > \text{Cd} > \text{Co} > \text{Sr} > \text{Mg.}$$

$$\text{Al-oxide: Cu} > \text{Pb} > \text{Zn} > \text{Ni} > \text{Co} > \text{Cd} > \text{Mg} > \text{Sr.}$$

$$\text{Mn-oxide: Cu} > \text{Pb} > \text{Mn} > \text{Zn} > \text{Ni.}$$

Laboratory studies of metal partitioning also identified the incorporation of Cr, Ni, Co, Cu, and Zn into a neoformed surface precipitates. Depending on the reaction conditions, time, and sorbent phase present, a metal hydroxide, a mixed layered double hydroxide (LDH), or a

phyllosilicate forms at the mineral surface. Many studies have shown that sorption of metals, such as Ni, on an array of phyllosilicates and Al-oxide, could result in the formation of mixed metal–Al hydroxide surface precipitates that appear to be coprecipitates. These neoformed phase shares structural features common to those of the hydrotalcite group of minerals (Sparks, 2003).

Evidence on the sorption of metal cations on microorganisms has been reported. Free-living bacteria and their extracellular macromolecular products (e.g. fibrils) can accumulate metal ions and may have mineral coatings with bound metals on their surfaces (Beveridge, 1989; Jackson and Leppard, 2002 and references therein). The cell surfaces of all bacteria are largely negatively charged, containing different types of negatively charged functional groups, such as carboxyl, hydroxyl, and phosphoryl that can adsorb metal cations. Biosorption comprises a variety of processes, including ion exchange, chelation, sorption, and diffusion through cell walls and membranes all of which are dependent on the species used, the biomass origin, and solution chemistry (Gavrilescu, 2004). Differently from humic substances, besides the formation of *outer-sphere* and *inner*-sphere complexes on the cell wall, considered to be a passive uptake in biosorption, active uptake, which transports the metal ions into the cell, is another peculiar mechanism for scavenging trace elements by living bacteria (Vijayaraghavan and Yun, 2008).

2.2. Sorption of Anions

Anions sorb primarily to variable charge minerals (metal oxides and noncrystalline aluminum silicates, allophanes), carbonate, and at the edges of phyllosilicates (Cornell and Schwertmann, 1996; Kampf et al., 2000). Usually, they are not sorbed on soil organic matter, but certain elements (e.g. borate, arsenate, arsenite) are found to be bound to humic substances (McBride, 2000; Thanabalasingan and Pickering, 1986; Violante et al., 2008b, 2012). The content of organic matter in soils has been usually correlated with the B sorption. It has been demonstrated that HA amino groups, due to their positive charge, play an important role in the sorption of arsenic onto organic matter (Kampf et al., 2000). Indeed, some anions may bond indirectly to organic groups through a bridging with hydrolytic species of Al and Fe (Mikutta and Kretzschmar, 2011).

The sites at crystal edges of phyllosilicates may easily sorb elements in anionic form as phosphate, sulfate, arsenate, selenite, molybdate. Kaolinite,

halloysite, and chlorite have a much greater capacity to sorb anions than illite or montmorillonite does.

Sorption of anions onto variable charge minerals and soils varies with pH. With increasing pH values, within a certain range, sorption decreases (due to a decrease in the positive charge of minerals) or else increases to a maximum close to the pKa for anions of monoprotic conjugate acids and then decreases. Slope breaks have been observed at pKa values for anions of polyprotic conjugate acids (Hingston, 1981). Arsenite and selenite may be sorbed more easily at high pH values because they form weak acids at low pHs and consequently may be dissociated only in alkaline environments.

Ligands that are specifically sorbed, forming *inner-sphere* complexes, replace $-OH^-$ or $-OH_2$ groups from the surfaces of variable charge minerals. Ligands, which form *inner-sphere* complexes, such as phosphate, arsenate, arsenite (on Fe-oxides), molybdate, selenite, and polydentate chelating low-molecular mass organic ligands (LMMOLs, e.g. oxalate, citrate, tartrate, malate) or HA and fulvic acid (FA), may form different surface complexes on inorganic soil components: monodentate, bidentate–binuclear and bidentate–mononuclear complexes in different proportions depending on the pH and surface coverage (Sparks, 2003). Phosphate and arsenate have similar behavior in soils. Arsenite is not strongly retained by aluminous minerals, but it has a strong affinity for the surfaces of minerals containing Fe (mainly [hydro]oxides) (Goldberg and Johnston, 2001; Ona-Nguema et al., 2005; Violante and Pigna, 2002; Violante et al., 2008b). Specifically sorbed anions usually lower the PZC of metal oxides; thus, the PZC of a particular oxide may give rise to different values depending on the kind and extent of foreign ion sorption.

Molybdate is a tetrahedral oxyanion similar to phosphate and arsenate and may compete with phosphate and arsenate for sorption sites on soil mineral surfaces. Applying the triple-layer model to calculate the distribution of ionic species of molybdate on goethite, Zhang and Sparks (1989) found that this anion is sorbed forming an *inner-sphere* surface complex primarily as $FeMoO_4$.

Sorption of chromate is maximum at pH 3.0–6.0 on iron oxides and decreases rapidly at higher pH values. Modeling efforts for chromate sorption on hydrous oxides of Fe and Al as well as in soils suggest that chromate forms an *outer-sphere* complex on these minerals (Zachara et al., 1989). Fendorf et al. (1997) suggested that on the goethite surface chromate forms monodentate, bidentate, and bidentate–mononuclear *inner-sphere* complexes.

This anion has a smaller shared charge compared with arsenite and arsenate, creating a weaker bond on sorption and, consequently, exhibits a steeper reduced sorption at near-neutral pH values compared with arsenate (Grossl et al., 1997).

Among the LMMOLs, oxalate has received particular attention. According to Parfitt (1978), oxalate may be sorbed on goethite mainly forming binuclear complexes, but large amounts are sorbed less strongly as monodentate complexes to permit increased sorption. Monodentate and binuclear surface complexes were found to form at pH 3.5 on one soil containing kaolinite, gibbsite, and hydroxyl-interlayered vermiculite, whereas bidentate surface complexes were formed at pH 4.5 and 5.5 (Bhatti et al., 1998). Hanudin et al. (2002) demonstrated that oxalate and citrate were sorbed onto allophanic samples in bidentate and/or binuclear form, but the binuclear form is more stable for oxalate and the bidentate form is more stable for citrate.

Sulfate and selenate should be able to form only *outer-sphere* complexes (Zhang and Sparks, 1990). However, there is some evidence by XAFS and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy that sulfate can also be sorbed as an *inner-sphere* complex. Peak et al. (2001); Sparks (1999) and Turner and Kramer (1991) demonstrated that sulfate may be sorbed onto the surfaces of variable charge minerals, forming at all pH values, both *inner-sphere* and *outer-sphere* complexes, with the former becoming more dominant with decreasing pH and increasing sulfate concentrations. Selenate seems to have a behavior similar to sulfate (Peak and Sparks, 2002; Wijnia and Schulthess, 2000a). Finally, the halide anions, with the exception of fluoride, and organic monocarboxylic anions bond by *outer-sphere* electrostatic attraction and are sorbed only if variable charge minerals surfaces are positively charged.

Anions are also sorbed by anionic clays (LDHs), which are present in soils (e.g. "green rusts"), but their affinity for these sorbents is different from that on variable charge minerals (Violante et al., 2009a, 2009b and references therein).

Till today, detailed information on the sequence of affinity of different anions onto soil components is still obscure. Competition in sorption among different anions may give useful information.



3. EFFECT OF INORGANIC AND ORGANIC LIGANDS ON CATIONS SORPTION

In natural environments, inorganic and organic ligands can significantly alter interactions between polyvalent cations and soil components

and soils. Many factors such as pH, surface properties of the sorbents, the number of sites available for sorption, species, and concentration of the ligands and cations, the nature and charge of Me–L species in solution influence the sorption of cations onto soil components and soils in the presence of inorganic and organic ligands (Violante et al., 2008b, 2012).

Metal sorption may be either enhanced or inhibited by the presence of anions through different processes, including alteration of surface charge, site competition, formation of different complexes in solution, ternary complex formation. Ternary surfaces complexes may be classified as type A, in which the metal ion is bonded to both the sorbent and the organic or inorganic ligand, or type B, in which the ligand is bonded to the sorbent between the surface and the metal ion (Fig. 3.1). The structure of sorbed species determines their stability and the extent of metal ion partitioning from solution to solid phase (Ha et al., 2009a, 2009b).

3.1. Sorption onto Phyllosilicates and Organic Matter

Inorganic and organic ligands usually inhibit the sorption of metal cations on negatively charged sorbents, by forming stable soluble negatively charged complexes with the cations and lowering the free cation activity in solution. Chloride ions form complexes in soil solution with some trace elements (e.g. Hg, Cd). They tend to form various dissolved complexes such as $HgCl_2$, $HgCl_3^-$, and $HgCl^+$, unless the pH is high enough for OH^- ions to prevail (Jackson, 1998). It has been ascertained that increasing concentrations of Cl^- strongly inhibited Hg sorption onto quartz (Sarkar et al., 1999). Sulfate and phosphate reduce Hg(II) sorption on quartz due to the formation of $Hg(OH)_2SO_4^{2-}$, $Hg(OH)_2H_2PO_4^-$, and $Hg(OH)_2HPO_4^{2-}$ aqueous species (Sarkar et al., 1999). Many authors (Abollino et al., 2008; McBride 1991; Wu et al., 2003) also showed that various LMMOLs (e.g. phthalate, fulvate, ethylenediaminetetraacetic acid (EDTA), oxalate, malonate, tartrate, and citrate) inhibited at different degrees the sorption of metal cations onto phyllosilicates by forming negatively charged soluble complexes (Fig. 3.2).

Anions may also enhance under certain conditions metal sorption on clay minerals (mainly kaolinite) at low pH values and low ligand/metal molar ratios. Zhou et al. (1999) found that Cu sorption on kaolinite at pH 4.0 was only very slightly increased by increasing oxalate/Cu molar ratio from 0 to 0.1 and was then drastically reduced at higher ratios. Similar findings were obtained by Lackovic et al. (2004a, 2004b) while studying

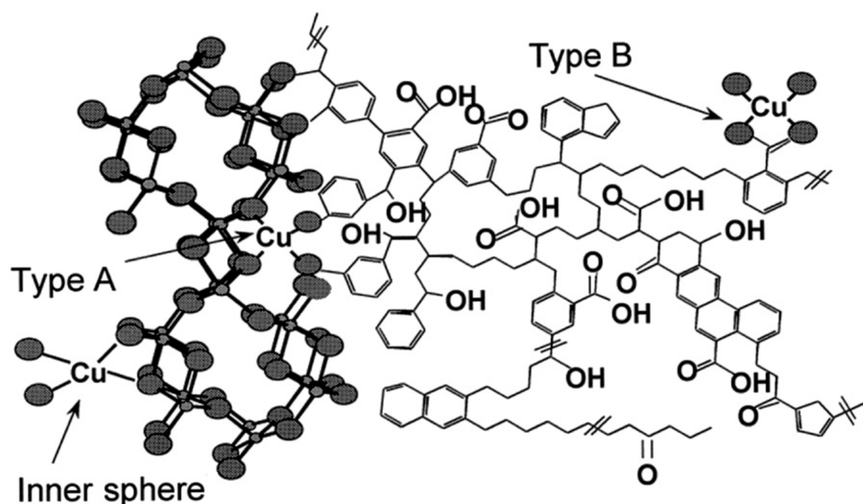


Figure 3.1 Illustration of some possible bonding configurations of Cu on goethite–humate complexes: *inner-sphere* complexation of Cu at goethite sites, and type-A- and B-ternary complexes. *(Reprinted with permission fromAlcacio et al. (2001)).*

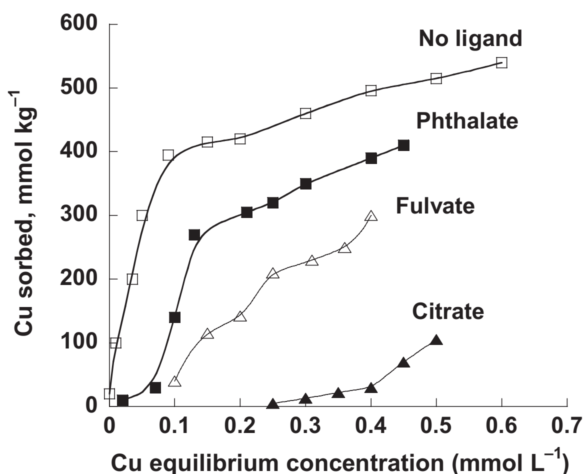


Figure 3.2 Effects of various organic complexing ligands on the sorption of Cu by montmorillonite. *(Modified from McBride (1991)).*

the effect of citrate on the sorption of Cd onto kaolinite and illite in the presence of citrate. Cadmium sorption on both these sorbents was slightly enhanced up to pH 5.0 in the presence of citric acid, but it was drastically suppressed at pH > 5.0. Sorption and potentiometric data were fitted by simple extended constant-capacitance surface complexation models.

Enhancement of sorption at pH < 5.0 was attributed to the formation of the ternary complex:



$$[X^-\text{--}K^+] + Cd^{2+} + L^{3-} + 2H^+ = [X^-\text{--}(CdLH_2)^+] + K^+$$

involving *outer-sphere* complexation with permanently charged negative sites on the faces of the minerals, whereas the suppression found between pH 5.0 and 8.0 was attributed to the formation of a strong negative complex (CdL^-), which did not sorb either on the permanent charged negative sites nor onto the surface hydroxyl groups at the particle edges. At higher pH values, the dominant species in solution were $CdLOH^-$, which were sorbed as *outer-sphere* complexes onto FeOH sites on the illite surface but not onto the AlOH sites on kaolinite.

Taylor et al. (2009) found that presorbed phosphate significantly enhanced the sorption of Pb on kaolinite in the pH range from 4.0 to 8.0. Based on the Pb-L3 edge XANES spectra, the product that forms on the kaolinite surface seems to have a composition and chemical environment similar to that of pyromorphite [$Pb_5(PbO_4)_3$ (OH, Cl)].

By using in situ resonant anomalous X-ray reflectivity, Lee et al. (2010, 2011) studied the role of FA in modifying the sorption mode of divalent cations (Ba, Sr, Pb, Hg, Cu, and Zn) onto the muscovite (001) surface. They found that in the absence of interfering ligands, three forms of sorbed cations were found: the classical *inner-sphere* and *outer-sphere* complexes and an "extended" *outer-sphere* complex located above the surface hydration layer or maintaining intact higher-order hydration shell (Lee et al., 2010; see their Fig. 3.2). For less strongly hydrated cations, the presence of FA increased metal sorption in the order Ba ≈ Sr < Pb < Hg, in sequence of increasing cation affinity for FA. Cations with a smaller affinity for FA tend to bind electrostatically on both the surfaces of muscovite and on the negatively charged functional groups of the organic matter, and in soils, they may be easily replaced by background cations as Ca and Na. Moderately hydrated cations with a greater affinity for organic matter may sorb more strongly on organic matter and are less exchangeable particularly at low pHs, whereas at higher pH values, these metal–organic complexes are released in solution for electrostatic repulsion between the surfaces of muscovite and the sorbed FA. Finally, the binding of strongly hydrated metals, such as Cu and Zn, onto the surfaces of muscovite was not altered by FA.

The presence of certain foreign ligands, occurring naturally in the rhizosphere, such as siderophores produced by microorganisms and phytosiderophores exuded by plants, may affect the sorption of metal cations onto phyllosilicates. Neubauer et al. (2000, 2002) studied the influence

of desferrioxamine-B (DFOB) on the sorption of Cu, Zn, and Cd onto montmorillonite and kaolinite. The presence of DFOB promoted, at certain pH values and DFOB/Me molar ratios, the sorption of the trace elements (Fig. 3.3A). Electrostatic interactions between the negative surfaces of phyllosilicates and the DFOB-Me positive complexes are the reasons for enhanced metal cation sorption onto montmorillonite (up to pH 8.0 for Zn). Shirvani et al. (2006) also demonstrated that DFOB siderophore enhanced the rate of Cd uptake by palygorskite, sepiolite, and calcite.

The influence of chelating organic ligands on the sorption of polyvalent cations onto HA and organo-mineral complexes has received attention. Usually, they compete with humic substances and reduce cation sorption. Wu et al. (2003) found that the amount of Pb sorbed onto an HA decreased with increasing concentration of EDTA, clearly because EDTA very strongly complexes with Pb ions and prevents their fixation on HA. Surprisingly, these authors found that increasing concentrations of citric acid promoted and not inhibited Pb sorption onto HA, but they did not give any explanation of their findings. However, it is possible to hypothesize that, as reported by Piccolo (2002), the addition of hydroxycarboxylic acids may modify the conformational association of humic substances with a disruption of supramolecular associations into smaller-size conformations, which facilitated the sorption of metal sorption.

Sorbed humic substances change the sorption of a cation at the mineral–water interface. As reported by Heidmann et al. (2004) "the specific interactions between metal cations and sorbed organic substances at mineral surfaces are not well understood, and partially contradicting results have been reported in the past. Several factors may play a role: (i) addition of high-affinity binding sites by organic functional groups, (ii) competition for reactive binding sites at the mineral surface, (iii) formation of ternary metal–organic surface complexes, (iv) alteration of surface charge and distribution of surface potentials, and (v) fractionation of HA substances during sorption to mineral surfaces. Sorbent interactions in mixed mineral–organic systems may lead to different nonadditive effects on metal sorption, depending on the nature and concentration of the metal cation, the pH, and the surface chemistry of the mineral and organic sorbents" (see Section 3.2.2).

Finally, the sorption of cations onto bacteria as affected by HA and other chelating ligands also received attention (Wightman and Fein, 2001; and references therein).

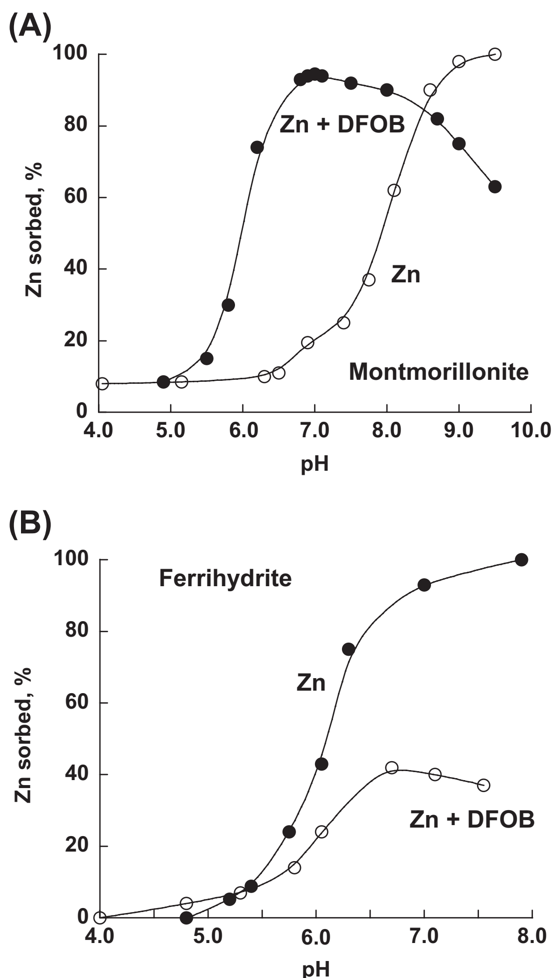


Figure 3.3 Sorption of Zn as a function of pH in the absence and presence of the hydroxamate siderophore DFOB on (A) montmorillonite (Zn concentration 87 μmol L^{-1}) and (B) goethite (Zn concentration 10 μmol L^{-1}). *(Modified from Neubauer et al. (2002)).*

3.2. Sorption onto Variable Charge Minerals and Soils

Organic and inorganic anions may interact directly with the surfaces of variable charge minerals or dissolved metal ions and may thereby alter the sorption/desorption processes of metal ions. In particular, the sorption of metal cations in the presence of inorganic and organic ligands onto the surfaces of variable charge minerals may be affected by several processes, which may occur simultaneously: (i) increased retention of metal cations

for the decrease of the positive charge of the mineral when a ligand is sorbed, (ii) increased retention due to the formation of negatively charged complexes between a metal and a ligand, which have a higher affinity for the sorbent, (iii) decreased retention due to the block of sorption sites by foreign ligands on the minerals, (iv) decreased retention due to the strong complexation of metal in solution in nonsorbing form, with lower free metal activity leading to desorption of surface-bound metal, (v) dissolution of the mineral in the presence of relatively high concentration of chelating organic ligands, leading to lesser metal retention (Cornell and Schwertmann, 1996; Sparks, 2003; Violante et al., 2008b and references therein). In each of the cases described before, the effect of a ligand on the sorption of metal cations depends not only on the strengths of the interactions between metal ion, ligand, and sorbent but also on their concentrations. Certainly, the initial ligand/metal molar ratio, the nature of the ligand and the metal, the surface properties of the sorbent seem to be critical in determining whether the sorption of a cation is enhanced or inhibited. Further, because anionic ligands are usually sorbed more strongly at low pH, whereas metal cations are fixed more easily at high pH, the same ligand may enhance the sorption of a given metal under some conditions but may suppress it under others.

The formation of sorbent–metal–ligand (type A) or sorbent–ligand–metal (Type B) ternary complexes on these minerals have been suggested (Schindler, 1990) (Fig. 3.1).

3.2.1. Effect of Inorganic Ligands

Many studies have been carried out on the effect of inorganic ligands (mainly sulfate and phosphate) on the sorption of metal cations onto variable charge minerals.

Enhanced sorption has been observed for Cd, Cu, Pb, Cd, and Zn on Al- and Fe-oxides in the presence of sulfate or phosphate (Bolland et al., 1977; Collins et al., 1999; Kuo, 1986; Venema et al., 1997; Wang and Xing, 2002, 2004) due to the increased negative charge brought to the surfaces by these inorganic ligands. Precisely, Collins et al. (1999) studied the coordination of Cd sorbed on goethite in the presence of phosphate, sulfate, oxalate, and citrate by using EXAF spectroscopy. These authors revealed that in the presence of phosphate and sulfate, Cd was *inner-spherically* bound most likely by bidentate double-corner sharing to adjacent terminal O groups on the (110) surface of goethite. They concluded that sulfate and phosphate sorbed to sites other than those occupied by Cd and that the enhancement of the

extent of Cd sorption was solely by electrostatic interactions and that no ternary complexes were formed. In contrast, the increase in the uptake of Pb onto goethite observed in the presence of sulfate and carbonate has been explained by the formation of type A ternary complexes, which are bound as bridging bidentate complexes to two adjacent surface oxygen [(═Fe–O)$_2$–Pb–OSO$_3$)] (Ostergren et al. (2000a, 2000b)). According to Ostergren et al. (2000a), the effects of carbonate on Pb sorption onto Fe-oxides may be negligible with P_{CO_2} at or near atmospheric concentrations but may significantly enhance Pb sorption in high CO_2 environments such as soils (up to 20% at 1 atm P_{CO_2} at pH 5.0).

Based on EXAFS and ATR-FTIR spectroscopies and batch sorption data, Elzinga et al. (2001) and Zhang and Peak (2007) proposed a mixture of ternary complexes and electrostatics to explain increased sorption of Pb (Fig. 3.4) and Cd, respectively, onto goethite. According to these authors, in addition to ternary complexes, Pb and Cd bound to goethite forming *inner-sphere* complexes that created additional positive charge, which favored sulfate sorption as both *inner-* and *outer-sphere* complexes. The relative impacts of these mechanisms (ternary complex and electrostatic effects) depended on pH values and Pb or Cd concentrations. Ternary A-type complexes were favored at low pHs and high Pb or Cd concentrations (Fig. 3.4B), whereas electrostatic effects predominated at high pH values and low metal concentrations (Fig. 3.4A). Elzinga et al. (2001) also demonstrated that sulfate initially sorbed on goethite surfaces forming *inner-sphere* complexes was partially transformed into Pb–SO_4 ternary complexes, particularly at low pH and high Pb concentration.

Other spectroscopic studies also indicated that goethite–M^{2+}–SO_4 (M^{2+} being Cu, Pb, Cd, and Zn) ternary complexes were of type A structure (Swedlund et al., 2009). Complexes with the same or similar structure were formed on ferrihydrite (Swedlund et al., 2003). Swedlund et al. (2003, 2009) suggested that ligands (e.g. phthalate) having stronger ligand–metal solution complexes also form stronger surface ternary complexes. Conversely, Beattie et al. (2008) demonstrated that ternary complexes between Cu, sulfate, and goethite were not formed under the conditions used in their study, their formation being possible only at high sulfate concentrations ($>2.5\cdot10^{-4}$ mol L^{-1}) and sulfate/Cu molar ratio > 1. Copper increased the affinity of sulfate for goethite, and Cu sorption edge shifted to lower pHs in the presence of sulfate due to a decrease in the electrostatic repulsion between the surfaces of the sorbent and Cu. Importantly, Cu ions altered the balance of *inner-* and *outer-sphere* sorbed sulfate (Fig. 3.5).

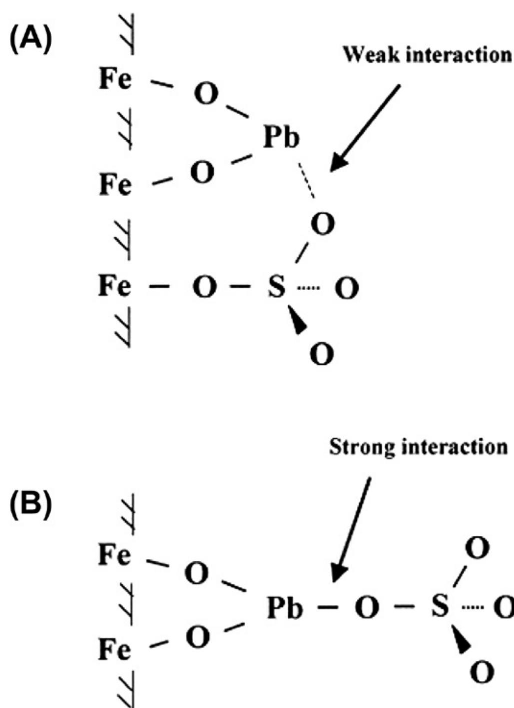


Figure 3.4 Pb–SO_4–goethite ternary complex configurations consistent with the FTIR and EXAFS data. *(Reprinted with permission from Elzinga et al. (2001)).*

Selenate did not affect Co sorption onto γ-Al_2O_3, whereas selenite significantly influenced Co sorption/desorption on/from the mineral (Boyle-Wight et al., 2002).

Weesner and Bleam (1998) demonstrated by XAS and electrophoretic mobility that sulfate and phosphate generally increased the sorption of Pb at the oxide–water interface of boehmite and goethite by different mechanisms. Surface-bound phosphate on both the metal oxides formed lead phosphate surface precipitates (pyromorphite), whereas sulfate ions were more effectively sorbed when Pb was present and enhanced Pb sorption on boehmite. Recently, Zaman et al. (2009) found that the sorption of Cd on manganese dioxide (β-MnO_2) was furthered by the presence of phosphate. The formation of type A ternary complexes was the main mechanism at lower pH, while the formation of type-B-ternary complexes was the predominant mechanism at higher pH values.

Studies on Cd sorption on goethite in the presence of phosphate (Venema et al., 1997; Wang and Xing 2002) evidenced that phosphate

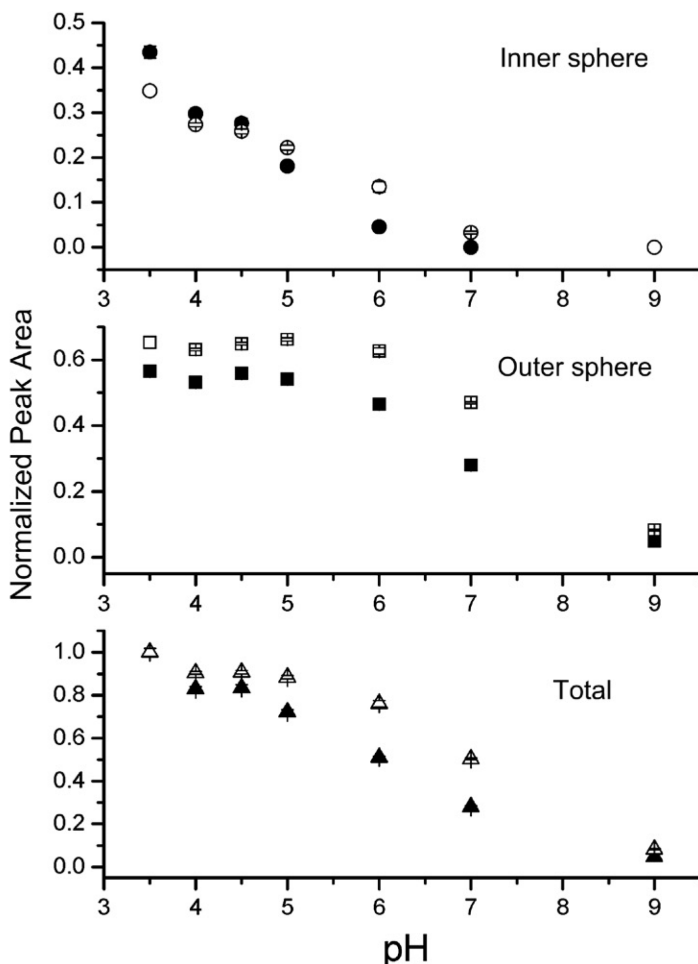


Figure 3.5 Normalized peak areas of the asymmetric stretching mode, ν_3, for *inner-sphere* (top), *outer-sphere* (middle), and total (bottom) bound sulfate, determined as a function of pH for sulfate sorption on goethite, and in the absence and presence of Cu. Filled symbols: $[K_2SO_4]$ 1×10^{-4} mol L^{-1}; [KCl] 1×10^{-2} mol L^{-1}. Unfilled symbols: $[K_2SO_4]$ 1×10^{-4} mol L^{-1}; [KCl] 1×10^{-2} mol L^{-1}; $[CuCl_2]$ 1×10^{-4} mol L^{-1}. *(Reprinted with permission from Beattie et al. (2008))*.

promoted not only Cd sorption (Fig. 3.6) but it also accelerated the sorption process (Wang and Xing, 2002). Cadmium was more easily desorbed from phosphate-treated goethite, probably because phosphate blocked mesopores and micropores of the Fe-oxide, causing fast sorption and desorption. According to Venema et al. (1996, 1997), the 021 face of goethite is important at low Cd surface coverage, while the

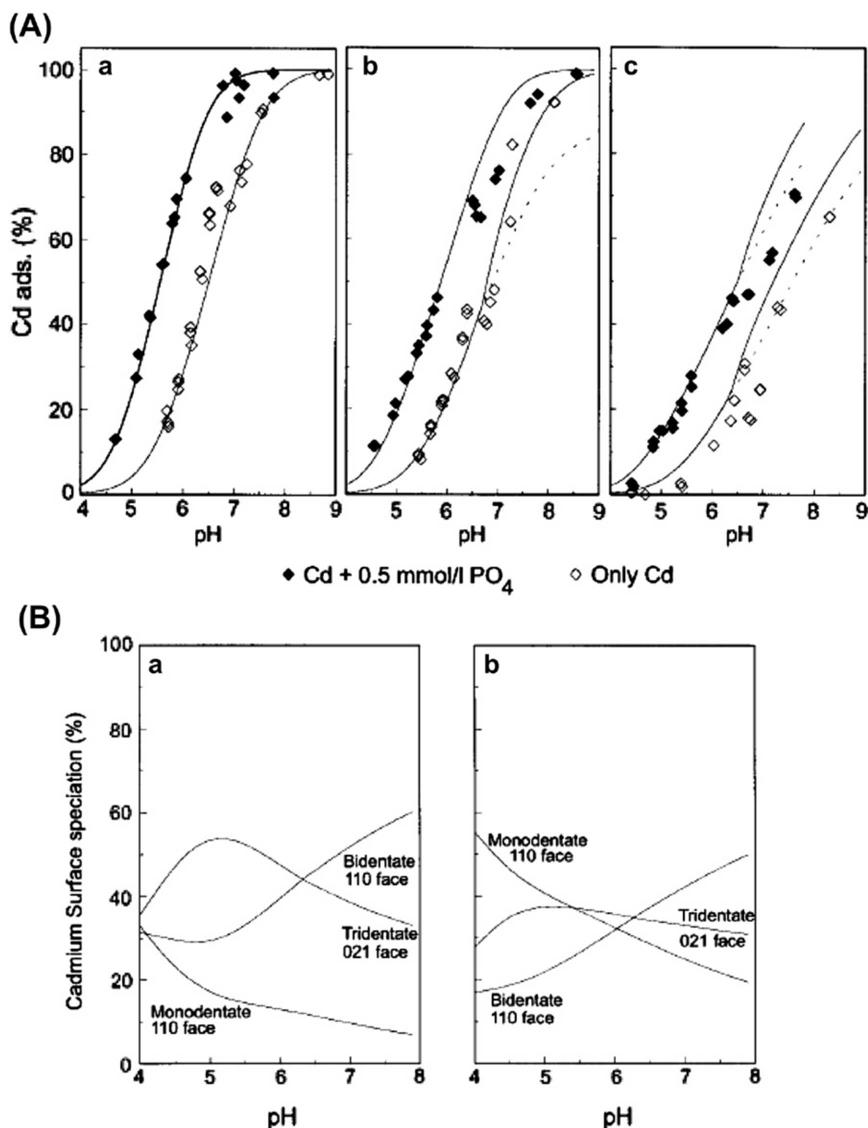


Figure 3.6 (A): Cadmium sorption data for goethite (570 m^2 L^{-1}) in 0.1 mol L^{-1} $NaNO_3$ with 0.25 mmol L^{-1} Cd (a), 0.5 mmol L^{-1} Cd (b), and 1.0 mmol L^{-1} Cd (c). The closed symbols represented data with 0.5 mmol L^{-1} phosphate, and the open symbols represent a system without phosphate. The solid lines represent the model calculations with CO_3. The dashed lines show the model calculation without carbonate species. In the model calculations, Cd monodentate surface complex (110 face) becomes more important when phosphate is present in the system. (B) Surface speciation of Cd on the goethite surface according to the model (CD-MUSIC) calculations. The calculations have been performed for a system with an ionic strength ($NaNO_3$) of 0.1 mol L^{-1}, 570 m^2 L^{-1}, goethite, and 1.0 mmol L^{-1}, Cd. Situations without (a) and with (b) 0.5 mmol L^{-1}, phosphate are considered. *(Reprinted with permission from Venema et al. (1997)).*

110 face dominates Cd sorption at high Cd coverage. These authors demonstrated using the CD-MUSIC model that the Cd monodentate surface complex (110) face becomes more important in the presence of phosphate (Fig. 3.6B). In contrast, Krishnamurti et al. (1999) reported a decrease of Cd sorption in the presence of phosphate in soils, which was attributed to the formation of Cd–phosphate complexes in solution, whereas McBride (1985) demonstrated that high levels of phosphate sorption on a noncrystalline metal oxide inhibited Cu sorption, probably due to the blocking of surface sites. Using hematite as sorbent, Li et al. (2006) found that phosphate pretreatment suppressed the maximum sorption of Cd and Cu, because phosphate occupied *inner-sphere* sorption sites on the surfaces of the sorbents, decreasing *inner-sphere* sorption sites for metals. Of course, the sorption of phosphate and other ligands with a strong affinity for the surfaces of variable charge minerals also increases the surface negative charges and decreases the electrostatic potential near the solid surfaces (Wang and Xing, 2004). However, it is possible to say that if metal cations are sorbed onto a sorbent mostly forming *outer-sphere* complexes, their sorption could be increased by the presence of phosphate (and other strongly chelating ligands), whereas, if they form *inner-sphere* complexes, the presence of phosphate anions, which also form strong *inner-sphere* complexes, suppresses their sorption.

Many authors have also reported that the sorption capacity of goethite toward anions increases with the incorporation of metal ions into its structure (Mohapatra et al., 2006; Mustafa et al., 2008, 2010) or hydroxyl apatite (Liu et al., 2010). Sorption of phosphate and chromate was found to increase substantially with Ni ion doping in goethite, due to an increase in the surface area and PZC of the sorbent (Mustafa et al., 2008, 2010). Mohapatra et al. (2006) observed that the PZC, the surface area, and the loading capacity of goethite increased with the increase of dopant percentage. These authors also found that the percentage of sorption of arsenate decreased in the order Cu-doped goethite ≥ Ni-doped goethite > Co-doped goethite > pure goethite, following the affinity of the metals for Fe-oxides (as discussed before in Section 2.1).

3.2.2. Effect of Organic Ligands

The formation of sorbent–metal–ligand (type A) or sorbent–ligand–metal (type B) ternary complexes has been suggested as the main mechanisms also

for the promotive effects of organic ligands on the sorption of metal cation onto variable charge minerals. McBride (1985) studied the sorption of Cu on microcrystalline gibbsite and boehmite in the presence of glycine (gly) at a gly/Cu molar ratio of 2.0 and 5.0, using ESR. Cupper formed a ternary complex in which the heavy metal coordinated simultaneously with a surface hydroxyl and at least one glycine molecule. Sorption of $Cu(gly)^{+}$ and $Cu(gly)_2$ species was favored on gibbsite and boehmite, respectively. Lenhart et al. (2001) examined the sorption of Pb onto hematite in the presence of malonic acid (malonate/Pb molar ratio = 10) combining EXAFS and ATR-FTIR spectroscopic techniques. Two types of Pb-malonate (type A) complexes were identified, a bidentate complex between Pb and both oxygen on malate, forming a four-membered ring, and a chelate involving a single oxygen from each of the carboxylate group of the organic ligand, forming a six-membered ring. The presence of both these complexes was suggested (Fig. 3.7), because the Pb–C distances (2.98–3.14 Å) were too short to be solely attributed to the formation of a six-membered ring and too long for solely four-membered ring complexes.

Boily et al. (2005) studied by using EXAFS and Infrared spectroscopy and potentiometric titrations the complexation of Cd–phthalate at the goethite/water interface and showed the formation of an *inner-sphere* Cd–phthalate complex on the (001) and an *outer-sphere* Cd–phthalate complex on the (110) plane of goethite. In both the complexes, Cd seemed to be in *inner-sphere* coordination with the surfaces of goethite. These authors

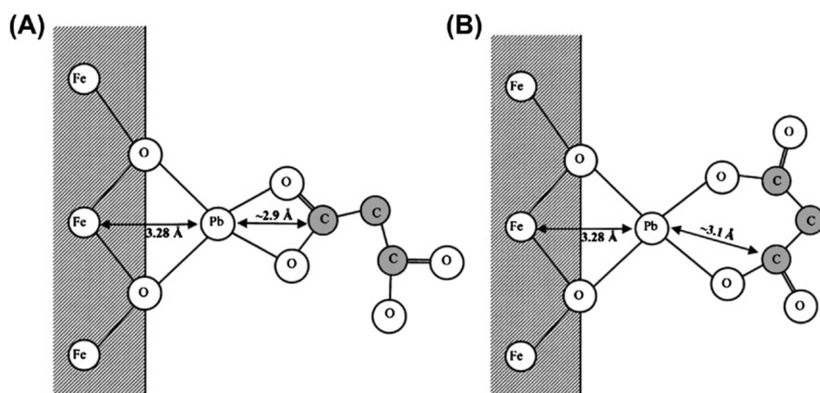


Figure 3.7 Schematic illustration of the proposed ternary Pb-malonate surface complexes. In this diagram, the distance for Pb–Fe is characteristic of a bidentate edge-sharing complex. (A) Malonate binds the Pb ion with two oxygens from one carboxyl group, forming a four-membered ring. (B) Malonate binds the Pb ion with one oxygen from each carboxyl group, forming a six-membered ring. *(Reprinted with permission from Lenhart et al. (2001)).*

claimed that the formation of type B complexes was not significant in the H–Cd(II)–Phthalate–FeOH system. Conflicting mechanisms were previously proposed for Pb-, Cu-, and Cd–phthalate co sorption where both ternary surface complexes of type A (Boily and Fein, 1998; Song et al., 2008) and/or type B complexes (Angove et al., 1999; Ali and Dzombak, 1996a, 1996b) were formulated. Fitts et al. (1999) studied by EXAFS and FT-IR the composition and the mode of sorption of Cu complexes on γ-Al_2O_3 in the presence of glutamate (glutamate/Cu molar ratio of 3). In the presence of this ligand, Cu sorption was enhanced at pH < 5.8 and inhibited at pH > 5.8 relative to the glutamate-free system (Fig. 3.8A), whereas glutamate sorption was always enhanced in the range of pH studied. Further, these authors found that in acidic systems, a 1- to 2-Cu-glutamate *outer-sphere* type B complex was the dominant species. In this type B complex, Cu was bonded to amino acid head groups of two

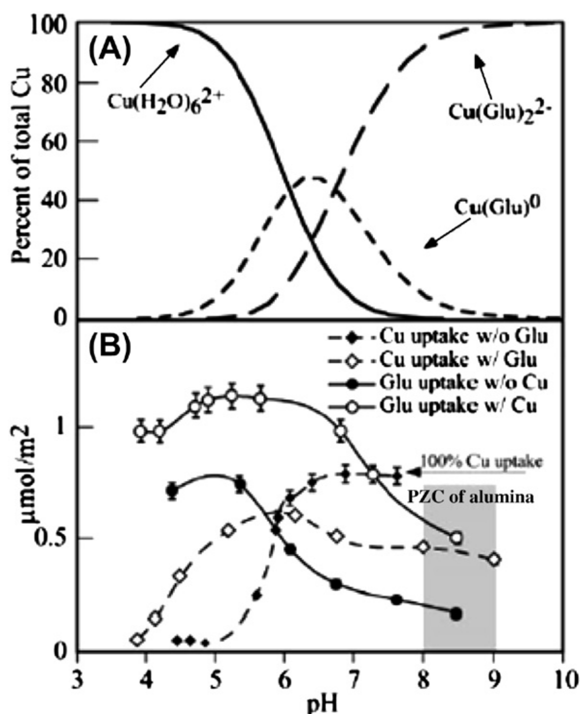


Figure 3.8 (A) Distribution of Cu-glutamate species in solution. (B) Cu uptake in the (1) absence and (2) presence of glutamate (Glu), and glutamate uptake in the (3) absence and (4) presence of Cu. Solution modeled assuming [Cu] = 0.5 mmol L^{-1}, [glutamate] = 1.5 mmol L^{-1}, 0.1 mmol L^{-1} $NaNO_3$. The shaded area shows the range of PZC values for γ-Al_2O_3. *(Reprinted with permission from Fitts et al. (1999)).*

glutamate molecules. In alkaline suspensions a 1- to 1-Cu-glutamate *inner-sphere* type A complex was the dominant species.

The sorption of Cu in the presence of an organophosphorus herbicide (glyphosate) on goethite was studied by batch sorption and ATR-FTIR and EXAF spectroscopies (Sheals et al., 2002, 2003). The system was investigated in the pH range 3.0–9.0. At pH 4, a ternary surface complex was identified with the phosphonate group of glyphosate forming a *inner-sphere* mono-dentate complex on the surfaces of the Fe-oxide, while carboxylate and amine groups coordinated to Cu to form a 5-membered chelate ring, with a type-B structure goethite–glyphosate–Cu. At pH 9.0, the phosphonate group was not coordinated, whereas the carboxylate group was coordinated to Cu with a different ternary structure goethite–Cu–glyphosate.

The nature of organic ligands also affects the sorption of metal cations and the nature of the complexes, as evidenced by Buerge-Weirich et al. (2003). These authors demonstrated that Cu sorption in the presence of oxalate (at $pH < 6.0$) or salicylate (at $pH > 5.0$) on goethite could be explained by a ternary complex of type A, whereas pyromellilate (pyromellic acid 1,2,4,5-benzene carboxylic acid) in acidic systems promoted the sorption of Cu and Cd by promoting the formation of ternary complexes of type B. Evidence for *inner-sphere* pyromellilate surface complex at $pH < 6$ has been demonstrated (Boily et al., 2000). Pyromellilate adsorbs on the surfaces of goethite by 2 carboxylic groups, so the formation of ternary surface complexes of type B is possible, because the two carboxylic groups on the opposite side of the ring can form a complex with metals. Ternary surface complexes of the type B are impossible with salicylate because of the unfavorable position of the functional groups. Salicylate sorbs by forming a six-membered ring, which involves one oxygen from the carboxylic groups, one from phenolic groups, and one Fe atom from the surface, so no functional group remains free to complex with a metal.

Angove et al. (1999) studied the effects of benzene carboxylic acids on the sorption of Cd by goethite and kaolinite. The acids investigated were phthalic (benzene-1,2-di-carboxylic acid), hemimellitic (1,2,3-tri), trimellitic (1,2,4-tri), trimeric (1,3,5-tri), pyromellitic (1,2,4,5-tetra), and mellitic (1,2,3,4,5,6-hexa) acids. These authors found that the influence of these carboxylic acids on Cd sorption on goethite and kaolinite depends on the extent to which they sorb to the sorbents, their capacity to complex with Cd, and the position of carboxylic groups around the benzene ring. Ligands that form strong complexes have the greatest effect on Cd uptake, but high concentrations of some acids, especially mellitic acid, prevented Cd sorption onto kaolinite (as discussed below).

The effect of increasing concentrations of organic ligands on the sorption of metals on soil sorbents has been studied (e.g. Angove et al., 1999; Perelomov et al., 2011; Violante et al., 2008a, 2008b, 2012; Zhou et al., 1999). Figure 3.9 shows the amounts of Cu (25 mmol added per kg) sorbed at pH 5.0 in the presence of oxalic or citric acid. By increasing the initial organic ligand/metal molar ratio from 0 to 10, the sorption of the metal on the iron oxide initially increased and then decreased, as referred to its sorption when added alone. In the presence of organic ligands, the pH_{50} (the pH at which 50% of the metal is sorbed) changed on changing the initial organic ligand/metal molar ratio. pH_{50} for Cu in the presence of oxalic acid (OX) decreased from 4.90 to 3.87 on increasing the initial oxalate/Cu molar ratio from 0 to 4 and then increased to 4.70 at an oxalate/Cu molar ratio of 10. As discussed before, the initial increase of Cu sorption must be attributed to the decrease of the positive charge of goethite due to the sorption of oxalate or citrate as well as the formation of negatively charged Cu–L_x complexes, which were easily retained on the surfaces of the Fe-oxide. By a further increase in the concentration of the organic ligands, Cu sorption decreased because many sorption sites were blocked by oxalate or citrate ions and for the formation of strong complexes in solution in nonsorbing forms. Similar results were found by Angove et al. (1999) who studied the sorption of Cd onto goethite (Fig. 3.10) and kaolinite in the presence of increasing concentrations of benzene carboxylic acids at different pH values. These authors also found that pH_{50} for Cd decreased at mellitic acid/Cd molar ratios of 1 and 10 and then increased at a mellitic acid/Cd molar ratio of 100. As summarized by Angove et al. (1999), "at low concentration a metal is partly sorbed, occupying a small fraction of the available surface sites on a sorbent. If a small amount of a ligand, which binds strongly to both metal and substrate, is added more metal will be sorbed, held indirectly to the surface by the ligands. On the other hand if the ligand concentration becomes much higher than that needed to saturate the surface sites, so the most of the ligand is in solution, the greater part of the metal may be held in solution as soluble complexes, and hence the sorption is lessened. Furthermore, because anionic ligands tend to sorb to mineral surfaces more strongly at low pH, whereas heavy metals sorb more at high pH, the same ligand may enhance sorption of a particular metal under some conditions but suppress it under others".

According to Alcacio et al. (2001) when organic substances are pre-sorbed on a mineral, possible binding arrangements of a metal cation, include sorption on the mineral surfaces or on organic matter (forming a type-B-ternary complex) or a binding arrangement that involves cation

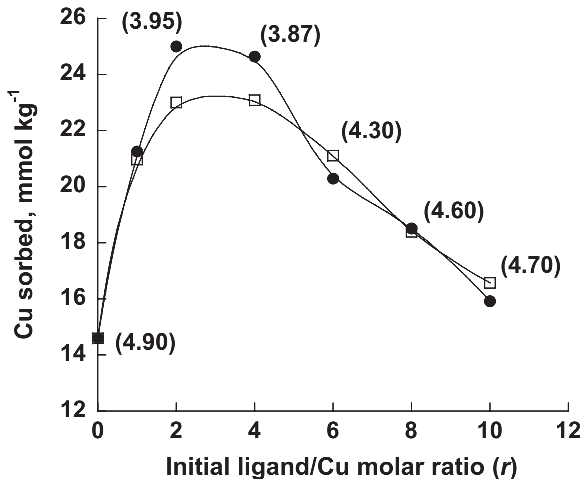


Figure 3.9 Sorption of Cu on goethite at pH 5.0 in the presence of increasing concentrations of oxalic (●) or citric (□) acid (initial ligand/Cu molar ratio from 0 to 10; 25 mmol of Cu added per kilogram of goethite). The numbers indicate the pH_{50} values of Cu sorbed in the presence of OX. *(Redrawn from Perelomov et al., (2011)).*

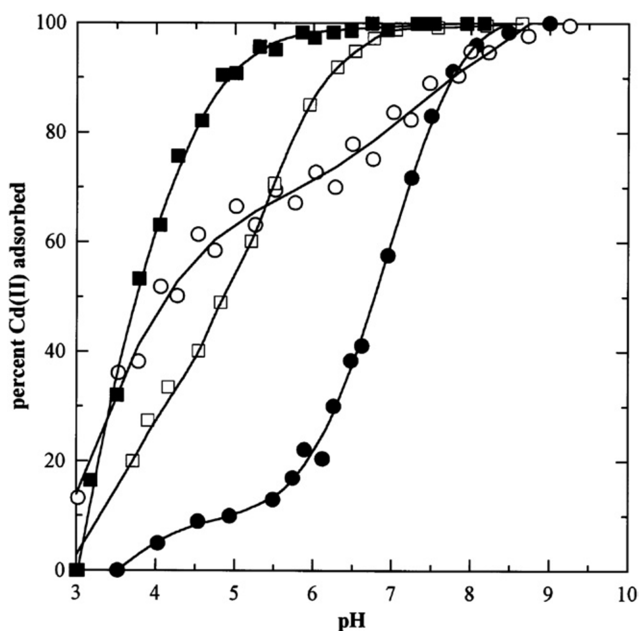


Figure 3.10 Cd sorption onto goethite in the presence of various concentrations of mellitic acid. Initial Cd concentration was 5×10^{-5} mol L^{-1}: (●) no added mellitic acid, (□) mellitic acid/Cd molar ratio of 1, (■) mellitic acid/Cd molar ratio of 10, (○) mellitic acid/Cd molar ratio of 100. *(Reprinted with permission from Angove et al. (1999)).*

bridging between clay and organic bonding sites (type A ternary complex). These authors using XANES and EXAFS data concluded that Cu was bonded on average to inorganic and organic sites as a complex when low amounts of humate covered the goethite surfaces and to organic functional groups as a type-B complex at higher levels of organic matter (Fig. 3.1).

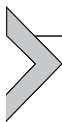
Neubauer et al. (2002) found that the sorption of Cu and Zn to ferrihydrite (Fig. 3.3B) and goethite was strongly affected by certain siderophores (DFOB), but their behavior was different from that of LMMOLs, as previously described (Figs 3.8–3.10). A strong mobilizing effect was observed for Zn (Fig. 3.3B). According to these authors, positively charged DFOB complexes were dominant up to pH 8.0 for Zn. Because the complexes are not attracted by the positively charged ferrihydrite, DFOB decreased the sorption of Zn (Fig. 3.3B), in contrast to montmorillonite suspensions (as discussed before; Fig. 3.3A).

The sequence of the addition of a metal and a ligand also affects the sorption of a metal. Violante et al. (2003) studied the sorption of Pb at pH 4.0 on the Fe-, Al-, and Fe–Al oxides in the presence of tartrate (tartrate/Pb molar ratio of 4) when Pb was added alone (*Pb*), as a mixture with tartrate (*Pb + TR* system), 30 min before tartrate (*Pb before TR* system) and 30 min after tartrate (*TR before Pb* system). It has been ascertained that on each oxide, the greater amounts of Pb were sorbed when tartrate was added before Pb and usually according to the following sequence: *Tr before Pb > Pb before Tr > Pb + Tr > Pb*. Certainly, by changing the order of addition of tartrate and Pb to metal oxides, different species were sorbed on the surfaces of the sorbents. These authors did not report spectroscopic studies of their samples. However, it is possible to hypothesize that in the *Tr before Pb* systems, the increased negative charge brought to the surfaces by previous sorption of tartrate anions promoted additional Pb sorption on the surfaces of the oxides, also by complexation with tartrate ions previously sorbed on the surfaces of the oxides, promoting type-B-ternary complexes. Vice versa, in the *Pb before Tr* systems, the previous sorption of Pb could increase the point of zero charge of the metal oxides, promoting the sorption of tartrate and some $PbTr_x$ complexes formed after tartrate addition, promoting both type-A and type-B-ternary complexes.

The formation of surface precipitates is also a possible mechanism that promotes the sorption of metal cations on variable charge minerals in the presence of organic ligands. Collins et al. (1999) demonstrated that the EXAFS spectra of sorbed Cd onto goethite in the presence of oxalate and

citrate at pH 5.3–5.4 were similar to that of Cd–oxalate and Cd–citrate and that the enhanced uptake of Cu by goethite was due to the formation of Cd–oxalate and Cd–citrate. The formation of surface precipitates were demonstrated by Grafe et al. (2004, 2008) using EXAFS when arsenate and Cu or Zn were cosorbed on goethite and jarosite.

Yamaguchi et al. (2002) studied the influence of surface area of gibbsite on Ni sorption in the ternary Ni–gibbsite–citrate system over time. At low surface area and corresponding high surface loading, a Ni–Al LDH precipitate formed. At high surface area and lower surface loading, the formation of an *inner-sphere* surface complex prevailed, and a small amount of Ni–Al LDH formed only after an extended aging period. Citrate reduced the amount of Ni sorbed, but the effect was more pronounced for the gibbsite with a low surface area than for that with a large surface area. Further, citrate prevented the formation of an LDH phase.



4. COMPETITIVE SORPTION OF CATIONS

In soil environments, multiple ions are present and compete for the surface sites of the sorbents. Competition in sorption between two or more metal cations is of paramount importance for understanding their relative affinity for a given sorbent.

4.1. Competition between Two Cations

The lack of competition of metal cations may be related to low surface coverage and/or precipitation effects and only partially to the presence of different binding sites having high selectivity for specific trace element ions (Kretzschmar and Voegelin, 2001; Violante et al., 2008b). Competition between metal cations for sorption sites of a sorbent has been demonstrated mainly at high surface loading of the sorbents with sorbed cations (Flogeac et al., 2007; Violante et al., 2008a; references therein). Kretzschmar and Voegelin (2001) reviewed the application of many surface complexation models to describe competitive sorption of metal cations for inorganic and organic soil components and soil materials.

Many factors such as the first hydrolysis constant, ionic radii, electronegativity and softness of metal cations and surface area, surface functional group, surface charge of sorbents have been proposed to explain the preference of the sorbent for the cations in competitive sorption. Studies conducted to understand competition in sorption of Zn, Ni, and Ca onto goethite (Trivedi et al., 2001) demonstrated that sorption affinity of these

cations followed the sequence Zn > Ni > Ca. In binary systems, Ni and Zn competition studies showed that at lower metal concentration when sites were not limited, no competitive effects were noted, whereas in the site saturation region, competition was observed. At different pH values in the site saturation region, the sum of the total sites occupied corresponded to the maximum number of sites ascertained from the single sorbate experiments. The single-site Langmuir model was able to describe sorption competition between these metals. In contrast, competition between Ca and Ni and Ca and Zn was negligible. Isotherms studies suggested that Zn and Ni (and other transition metals) bind to one type of site at high affinity, whereas Ca (and other earth cations) held to low affinity sites that comprise 100 x that of the high-affinity ones. Competition was not observed as Ca only sorbed to these sites. Similar findings were obtained by Heidmann et al. (2005) who studied the competition in sorption of Cu and Pb onto kaolinite over wide concentration ranges and used three different models based on surface complexation and cation exchange reactions. Two types of binding sites were assumed, edge sites with pH-dependent charge and face sites with permanent negative charge. Experimental data showed that Pb was held more strongly than Cu on kaolinite. As a consequence, Cu sorption was decreased in the presence of Pb due to competition for sorption sites. At pH > 6.25, the amount of Cu sorbed was not influenced by Pb. Model calculations suggested that at low pH values Pb and Cu were fixed mainly to face sites, whereas at high pH values, sorption to edge sites predominated.

To describe the sorption of Pb and Cd added alone and in binary systems on natural soils, Serrano et al. (2009) developed a nonelectrostatic equilibrium model with both surface complexation and ion exchange reactions for which surface site densities were estimated from mineralogical fractions in the soil. Soil heterogeneity was modeled with >FeOH and >SOH functional groups, representing Fe- and Al-oxides and edge sites of phyllosilicates and two different exchange sites (X^- and Y^-), to account for the ion exchange capacity attributed to smectite, illite, and kaolinite and organic matter. The model showed that metal sorption was dominated by exchange reactions on permanent charge sites at low pH, with a greater tendency of Cd to be retained on these sites. At higher pH, sorption of Pb and Cd on >FeOH sites, and to a lesser extent on >SOH sites, became more important, with Pb having a greater affinity than Cd for >FeOH sites. Lead sorption on >FeOH occurred at lower pH than for Cd, suggesting that Pb sorbs to surface hydroxyl groups at pH values at which Cd interacts only with exchange sites. Modeling of Pb and Cd sorption in binary

systems showed a great decrease of >FeOH sites occupied by Cd compared with Cd added alone at the same concentration, while the sorption of Pb on these sites did not change in the presence of Cd. Model results suggested competition for >FeOH and removal of Cd from hydroxyl sites to ion exchange sites in the presence of Pb.

Violante et al. (2003) demonstrated that Cu has a greater affinity than Zn for ferrihydrite surfaces and thus strongly inhibited the sorption of Zn on common sites at pH 4.5 and was also able to remove Zn previously sorbed onto them; vice versa, Cu sorption was not affected by the presence of Zn, even at an initial Zn/Cu molar ratio of 8. Similar results were found by Arias et al. (2006) and Saha et al. (2002). Violante et al. (2003) also studied the effect of residence time of Zn sorption onto ferrihydrite in the presence of Cu and at an initial Zn/Cu molar ratio of 2, when Cu was added from 1 to 336 h after Zn addition. Zinc sorption increased, particularly when Cu was added 6–336 h after Zn, clearly because increasing amounts of Zn ions initially added became less desorbable by Cu. As reported before, metal cations initially sorbed on the surfaces of variable charge minerals decrease in mobility with time due to diffusion and incorporation into the minerals. According to Fischer et al. (2007), solid-state diffusion into the interior of the particles of iron oxides leads to an immobilization of the metals. The first step of a reaction of a metal cation with an oxide is a very fast (few minutes) sorption onto the external surfaces, followed by a solid-state diffusion into the particles and sorption on the internal surfaces (e.g. micropores and voids). The diffusion process is very slow and may continue for many months with oxides and for several years with soils, depending on the binding strength and ionic radius of the diffusion ions.

Surface precipitation of new surfaces phases is also considered to be an important process, occurring on a time scale of hours to days. Clearly, in the experiments previously described, Cu added several hours or days after Zn addition did not replace Zn ions that were probably diffused into the micropores of the sorbent and/or had formed precipitates on the surfaces of the ferrihydrite (Violante et al., 2003). Fischer et al. (2007) claimed that the formation of new solid phases on the surfaces of the sorbents by heavy metals previously sorbed may occur only at relatively high concentrations (>0.007 μmol m^{-2}).

The effect of major elements (Ca, Mg, Al, and Fe) on the sorption of Cu, Pb, and Cr onto a montmorillonite at different pHs (3.5, 4.5, and 5.5) has been investigated by Zhu et al. (2011a). The sorption of Cu, Pb, and Cr in the presence of Ca and Mg generally decreased with

increasing Ca and Mg concentrations. The inhibition by both Ca and Mg for these heavy metals decreased with the increase of pH and followed the sequence of Pb > Cu >> Cr at pH 3.5 and Cu > Pb >> Cr at pH 4.5 and 5.5, confirming the more intense competition at lower pH and the strongest sorption of Cr on montmorillonite. The sorption of these metals on montmorillonite was also inhibited by Al and Fe when the pH and/or the concentration of Al and Fe was relatively low, whereas Al and Fe with higher concentration promoted the sorption of heavy metals especially at pH 4.5 and 5.5. Aluminum and Fe form monomers or small exchangeable polymers at low pH (Cornell and Schwertmann, 1996; Huang et al., 2002; Sposito, 1996; Violante et al., 2002). The inhibition of Ca and Mg as well as that of Al and Fe at low pH on the sorption of heavy metals by montmorillonite was due to their competition with heavy metals for the homogeneous sorption sites. By increasing the pH, Al and Fe easily form polymers and nanoprecipitates that are sorbed irreversibly on the external surfaces and in the interlayers of montmorillonite, forming chloritelike complexes. Heavy metals are selectively and strongly sorbed on AlOH– and Fe–OH species, which coat the surfaces of montmorillonite (Violante and Gianfreda, 2000; Violante et al., 2008a, 2008b, and references therein).

Competitive sorption of metal cations onto natural organic matter also received great attention. Kinniburgh et al. (1996) demonstrated that Cd significantly reduced Ca sorption onto a peat HA. In contrast, Cd sorption was also reduced by increasing Ca concentrations. However, using a peat HA containing a large quantity of phenolic functional groups, the influence of Ca in reducing Cu sorption was negligible, probably due to a much greater affinity of Cu for phenolic groups as compared to Ca.

Many researchers studied the influence of Ca and Mg on the sorption of heavy metals onto HA (e.g. Benedetti et al., 1996; Tipping, 1993) or FA (e.g. Kinniburgh et al., 1999; Mandal et al., 2000). Benedetti et al. (1996) demonstrated by using the new Nonideal Competitive Adsorption model that Cu competes much more efficiently with protons bound to the phenolic groups of an HA than Ca and Cd. Mandal et al. (2000) demonstrated that the presence of high concentrations of Ca and Mg (four orders of magnitude higher than those of Ni) have considerable effects on the sorption of Ni by FA. Calcium and Mg competed with Ni for sites where electrostatic interactions dominate resulting in Ni forming weak Ni–FA complexes that are labile. According to these authors, in freshwaters, the competition of Ca and Mg (in high concentrations) with Ni (present in trace quantities) for

binding sites produces weak Ni–humate complexes that are labile, releasing Ni ions.

Qin et al. (2006) studied the mechanisms of the competitive sorption of Pb, Cu, and Cd on peat HA in single and multisolute systems (Fig. 3.11) by the combined use of batch equilibration sorption and XAS. Peat contained lignin, cellulose, and humic substances as its major component, which had functional groups such as ketones; aldehydes; and carboxylic, hydroxylic, and phenolic groups. The sorption capacity and initial sorption rate in single-solute systems followed the order Pb > Cu > Cd. The sorbed amount of each metal (q_m') and the initial sorption rate were decreased in binary and ternary systems due to competitive effects. These authors ascertained that the sorbed amounts of metals at low energy sites ($q_{m,1}$') decreased substantially compared to those at high-energy sorption sites ($q_{m,2}$'), evidencing that competition sorption among Pb, Cu, and Cd occurred mainly for the sites at low energy. XAS spectroscopic study offered a direct evidence that Pb and Cd were coordinated to carboxylic groups and, probably, hydroxyl groups.

Other studies on the competition of two metal cations have been described by Kretzschmar and Voegelin (2001) and Violante et al. (2008a).

Few investigations have been carried out on the competition in sorption of two cations onto soil mineral–bacteria complexes. Recently, Zhu et al. (2012) studied the competitive sorption of Cu and Cr on a goethite-*B-thuringiensis* complex compared with that on goethite. The competition in sorption between these elements was more intensive on goethite-*B-thuringiensis* complex than on goethite, and the competitive ability of Cr was stronger than that of Cu on both the sorbents. These authors demonstrated that the affinity of metal cations for the goethite-*B-thuringiensis* complex was lower than that for goethite. Greater amounts of these trace elements were replaced by Ca from the complex than goethite.

4.1.1. Effect of Anions on Competitive Sorption of Cations

Competition in sorption between two metals in the presence of organic or inorganic ligands has received scant attention. Perelomov et al. (2011) studied the effect of increasing concentration of Pb on the sorption of Cu (initial Pb/Cu molar ratio ranging from 0 to 10) at pH 5.0 on goethite, in both the absence and presence of OX. OX was added as a mixture with the heavy metals (*Cu + Pb + OX* systems) or 1 h before Cu + Pb addition (*OX//Cu + Pb* systems). Lead strongly prevented Cu sorption, but its inhibition was affected by the initial Pb/Cu molar ratio and presence of OX

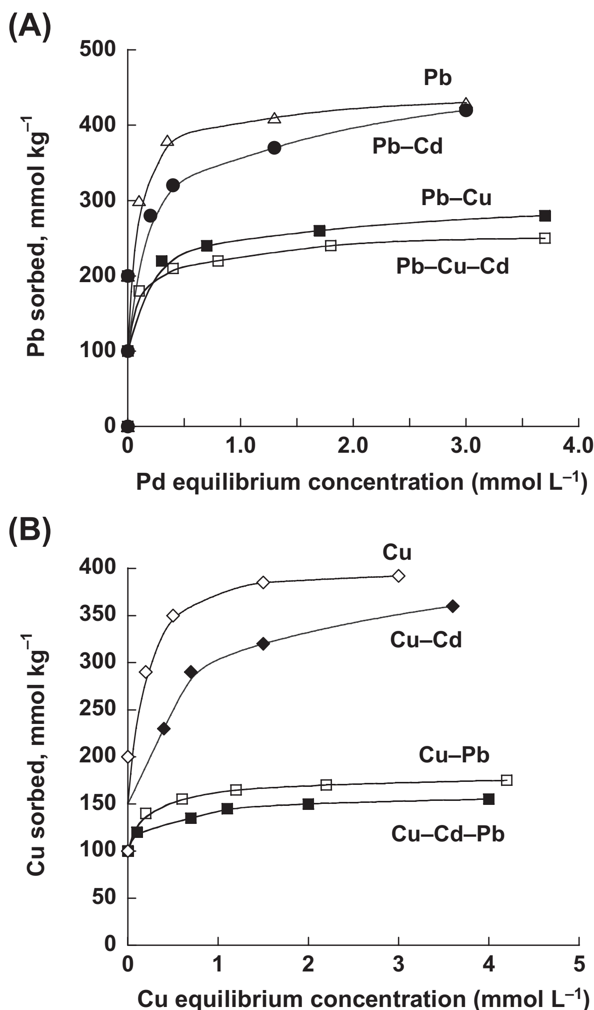


Figure 3.11 Sorption of Pb and Cu on Heilongjoing soil at pH 6.0 in single and multisolute (Pb, Cu, and/or Cd) systems. Solid lines represent Langmuir two-site model fitting. *(Redrawn from Qin et al. (2006))*.

(OX/Cu + Pb molar ratio of 2). In the absence of OX (*Cu* + *Pb* systems), the inhibition of Pb in preventing Cu sorption increased from 11% to 55% by increasing Pb/Cu molar ratio from 1 to 10 (Fig. 3.12). The presence of OX improved Cu sorption more when added together than before the metals. These authors also found that the final Cu sorbed/Pb sorbed molar ratio when equimolar amounts of the two heavy metals were added as a mixture in the absence or the presence of OX increased from 1.58 in oxalate free

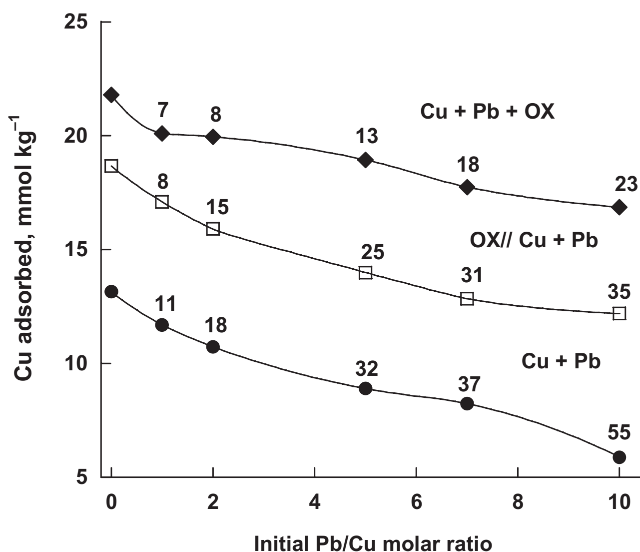


Figure 3.12 Sorption of Cu on goethite (25 mmol Cu per kilogram) at pH 5.0 in the presence of increasing concentrations of Pb (initial Pb/Cu molar ratio ranging from 0 to 10) and in absence or presence of OX (OX; 50 mmol OX per kilogram of goethite). OX was added as a mixture with Cu and Pb (*Cu + Pb + OX* systems) or 1 h before Cu and Pb (*OX//Cu + Pb* systems). Numbers on the curves indicate inhibition (%) of Pb and OX on Cu sorption. *(Redrawn from Perelomov et al. (2011))*.

system to 2.05 and 2.28 in *OX//Cu + Pb* and *Cu + Pb + OX* systems, respectively, indicating that the presence of OX promoted Cu fixation more than Pb.

In soil environments, mainly in the rhizosphere, LMMOLs, such as oxalic, citric, tartaric, and malonic acids and HA and FAs may interact with Fe and Al species and/or phyllosilicates (Huang, 2004; Violante and Gianfreda, 2000; Yuan and Theng, 2012) promoting the formation of organo-mineral complexes. Zhu et al. (2010) studied the competitive sorption of Cu, Cr, and Pb in binary and ternary systems on ferrihydrite and on an organo-mineral complex obtained by coprecipitating Fe with oxalate and tartrate. The competition in sorption of the heavy metals was affected by the nature and the concentration of the elements, the surface properties of the sorbents, the sequence of addition of the heavy metals, and the residence time. In binary systems, the highest inhibition capacity on all the sorbents was for Pb versus Cu, whereas the lowest was for Cu versus Pb. The inhibition of Pb for Cu was much stronger than of Cu for Cr. However, Cu competed with Cr and Pb more intensely on organo-mineral complexes than on ferrihydrite, whereas Pb competed with Cr more intensely onto ferrihydrite.

4.2. Competition among Three or More Cations

Only few studies have been carried out on the competition of three or more trace elements onto soil components or soils. Elliott et al. (1986) studied the competitive sorption of Cd, Cu, Pb, and Zn onto four soils with different chemical properties. For two mineral soils, sorption under acidic conditions (pH 5.0) followed the sequence Pb > Cu > Zn > Cd, which corresponds to the order of increasing pK for the first hydrolysis product. For two soils with high organic matter content, the order was Pb > Cu > Cd > Zn.

Sorption of Cd, Cu, Pb, and Zn onto kaolinite in single- and multicomponent systems has been studied also by Srivastava et al. (2005). The selective sequence of these metals in multielement systems was Cu > Pb > Zn > Cd. The sorption and potentiometric titration data for various kaolinite–metal systems were modeled using an extended constant-capacitance surface complexation model that assumed an ion exchange process at pH < 7.0 and the formation of *inner-sphere* surface complexes at higher pH values. This model proposed different reaction mechanisms. At low pH, all the metals sorb onto permanent charge sites by ion exchange reactions, whereas at higher pH, the metals form *inner-sphere* complexes on to variable charge sites at the crystal edges and octrahedral alumina faces of the mineral. The hydroxyl species of Cu and Pb form monodentate *inner-sphere* complexes, whereas Cd and Zn form bidentate complexes. Competition in sorption of metal cations in binary and ternary systems on hydroxyaluminum- and hydroxyaluminosilicate–montmorillonite complexes has been studied by Saha et al. (2002).

Qin et al. (2006) in their study on the competition in sorption on peat of Cu, Pb, and Cd in binary and ternary systems (Fig. 3.11) showed that at low initial metal concentrations, the competition in sorption was not pronounced, and vice versa when the metal concentration increased, the metals with the greater affinity strongly competed with metals with lower affinity for the sorption sites of the organic matter. When Pb, Cu, and Cd were added as a mixture, Pb and Cu competed with Cd, whose sorption was substantially reduced. In binary and ternary systems, the total sorption capacity of the metals increased, but individual metal sorption decreased. Capasso et al. (2004) showed similar findings while studying the sorption of Cr, Cu, and Zn on a humiclike substance. Although Cu and Zn were reduced in the presence of the other metals in binary or ternary systems, Cr(III) sorption was not affected by the presence of Cu and Zn.

Zhu et al. (2010) also studied the competition in sorption among Pb, Cr, and Cu added as a mixture on ferrihydrite and an organo-mineral complex. When equimolar amounts of each metal were added, the surfaces of ferrihydrite and organo-mineral complex were covered with >50% (53–56%) by Pb, with 32–36% by Cr, and with 9–13% by Cu. However, in the presence of large amounts of Pb and Cr (Pb:Cr:Cu molar ratios of 2:1:1, 1:2:1 or 2:2:1), some sites were still occupied by Cu even though Cu had a much lower affinity than did the other two metals for both the sorbents. These results indicated that a small proportion of the sites on both sorbents had a high affinity for Cu, particularly on the organo-mineral complex. A similar behavior was ascertained by Covelo et al. (2004) when these metals competed for sorption sites of four different HA umbrisols. Later, the same authors (Covelo et al., 2007) studied the simultaneous sorption and desorption of six heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) by kaolinite, vermiculite, mica, Fe- and Mn-oxides, and organic matter. Kaolinite and mica preferentially sorbed Cr, vermiculite sorbed Cu and Zn, HA substances and Fe- and Mn-oxides sorbed Pb and Cu.

5. COMPETITIVE SORPTION OF ANIONS

5.1. Competition for Sites and Effect of Changes in the Electric Potential

Many studies on the competition between two or more anions for the surfaces of clay minerals and soils have assumed that the only mechanism implied was competition for sites. In other words, the sorption of a ligand inhibits the sorption of the competing anion(s) only because it decreases the number of sites available on the mineral. Barrow (1992) demonstrated that competition is mainly through changes in the electric potential of the surface of the sorbent rather than through decreases in the number of vacant sorption sites. This author studied the competition between phosphate and selenite onto a soil. When added alone, these ligands continued to be sorbed through the entire period of reaction studied (1000 h) due to a diffusive penetration of the surface of the sorbent, but the continuing reaction was more evident for phosphate than for selenite. By adding equimolar amounts of these two ligands as a mixture, it was evident that the presence of a competing anion decreased the sorption of the other, but the effect was initially negligible but increased with time. Barrow (1992) concluded that the negligible effect of one anion on the other after short periods of reaction evidences that "chemical" competition for vacant sites was not important.

So, this author concluded that, because true *adsorption* would be expected to occur quickly, competition was due to changes in the electric potential of the surfaces of the sorbent.

Indeed, many studies have evidenced that competition in sorption between two ligands also occurs after a very short period of reaction (Barrow et al., 2005; Frankenberger, 2002; Violante and Pigna, 2002; Violante et al., 2005). This mechanism seems evident when the competing anions have a similar affinity for the surface of the sorbent, whereas the influence of electric potential is more important when competition occurs between ligands with a great different affinity for the sorbent (e.g. phosphate and sulfate, as discussed later).

Violante and Pigna (2002) and Violante et al. (2008a) studied the amounts of arsenate and phosphate sorbed onto noncrystalline Al-hydroxides after 0.03–168 h when these ligands were added alone or as a mixture at 50% (Table 3.1) of surface coverage. Each ligand inhibited the sorption of the other even after few minutes of reaction. Arsenate and phosphate were completely sorbed onto $Al(OH)_x$ within 3–5 h of reaction when added alone, but only after >168 h when added as a mixture (Table 3.1). When the oxyanions were added together, the arsenate sorbed/phosphate sorbed molar ratio (rf) continuously increased with time from 0.21 after 0.03 h to 0.94 after 168 h. The rf values were initially <1, because the sorption of phosphate was faster than that of arsenate. The rf reached the value of 1 only after 720 h (data not shown), indicating that all the ligands added were fixed on the surfaces of the oxide. In contrast, on ferrihydrite and hematite

Table 3.1 Kinetics of Reaction of Arsenate (AsO_4) and Phosphate (PO_4) onto $Al(OH)_x$ When Added Alone or as a Mixture ($AsO_4 + PO_4$; initial AsO_4/PO_4 molar ratio of 1) at 50% Surface Coverage

	AsO_4 and PO_4 added alone		$AsO_4 + PO_4$		
	AsO_4 sorbed	PO_4 sorbed	AsO_4 sorbed	PO_4 sorbed	
Time (hrs)	mmol kg^{-1}				rf = AsO_4/PO_4
0.03	143	162	32	154	0.21
0.5	166	196	60	157	0.38
1	186	219	81	198	0.41
3	234	236	111	225	0.49
24	250	245	198	244	0.81
48	250	249	224	241	0.92
168	250	233	232	247	0.94

rf indicates the AsO_4 sorbed/PO_4 sorbed molar ratio.
Two hundred fifty millimoles of AsO_4 added per kilogram of sorbent.

at 50% surface coverage of each competing ligands, the rf values were initially >1 and then decreased with time up to 1, because arsenate sorption was initially faster than that of phosphate, arsenate having a greater affinity for Fe than phosphate did (Frankenberger, 2002; Violante and Pigna, 2002; Violante et al., 2005). Clearly, an initial faster sorption of an anion onto the surface of a given sorbent affects the sorption of the other. A reduction in surface charge because of the initial sorption of phosphate or arsenate may also differently reduce the rate of anions sorption, which may be responsible for the observed residence time effect.

Later, Barrow et al. (2005) studying the competition between phosphate and selenite and phosphate and arsenate onto a Chilean Andisol concluded that competition between ions is not only competition for the occupancy of sites available onto the surfaces of the sorbents but it also involves a change in charge and then the electric potential of the surface due to sorption of ions and their subsequent penetration of the surface.

5.2. Competitive Sorption Involving Sulfate and Phosphate

It is well established that the capacity of a soil to sorb sulfate can be affected deeply by management practices, particularly by liming of acid soils or by the application of phosphate. Both these practices decrease sulfate sorption and facilitate its leaching (Curtin and Syers, 1990).

Many studies have been carried out on the competitive sorption of sulfate and phosphate on soils, phyllosilicates, and variable charge minerals. Several authors suggested that the mechanisms of sulfate and phosphate sorption on variable charge minerals and soils are similar and that both ions compete for the same sorption sites, although sulfate is sorbed less than phosphate is and does not compete strongly with phosphate (Pasricha and Fox, 1993; Sumner, 1993; and references therein). In fact, phosphate solutions are used to extract sulfate in soil tests for sulfur (Parfitt, 1978). Geelhoed et al. (1997) and Pigna and Violante (2003) demonstrated that when phosphate and sulfate were added in equimolar amounts to variable charge minerals or soils, the sorption of sulfate was largely decreased, whereas the effect of sulfate on the amount of fixed phosphate was small or negligible. The latter authors carried out experiments on the sorption of sulfate in the presence of phosphate (initial phosphate/sulfate molar ratio ranging from 0 to 1) at pH 4.5 on an Andisol sample containing 42% of allophanic materials (Fig. 3.13). Sulfate was added at its maximum surface coverage and phosphate added was completely sorbed on the sorbent. Sulfate sorption was strongly inhibited by the presence of phosphate, even at low phosphate/sulfate molar

ratios. In fact, at phosphate/sulfate = 0.1, sulfate sorption decreased by 67% compared with sulfate sorbed in the absence of phosphate. At phosphate/sulfate = 1 and 1.2, sulfate sorption was, respectively, reduced of 92% (Fig. 3.13) or completely inhibited. The decrease of sulfate sorption on soil sample could not be attributed to just direct competition between phosphate and sulfate because the sulfate decrease was much greater than the amounts of phosphate sorbed and many sites for phosphate sorption were available on the sample. Clearly, phosphate sorption decreased the surface charge and point of zero charge of the Andisol sample, preventing the fixation of sulfate ions on more negative surfaces (as discussed before; Barrow, 1992).

Experiments on sorption of sulfate on Andisol in the presence of increasing amounts of phosphate were carried out also at low pH values (pH 3.0, 3.5, and 4.5; Pigna and Violante, 2003). It was found that decreasing pH enhanced sulfate competition with phosphate. For example, at phosphate/sulfate = 0.2, phosphate inhibited sulfate sorption by 7% at pH 3.0 versus 11% and 46% at pH 3.5 and 4.5, respectively. These findings may be explained considering that sulfate may form strong complexes on the surfaces of variable charge sorbents at low pH values (say <5.0; Sparks, 1999; Turner and Kramer, 1991), which enhance the ability of sulfate to compete with phosphate and other anions. Many investigations also showed that the competitive effect of sulfate on other anion sorption (chromate, arsenate,

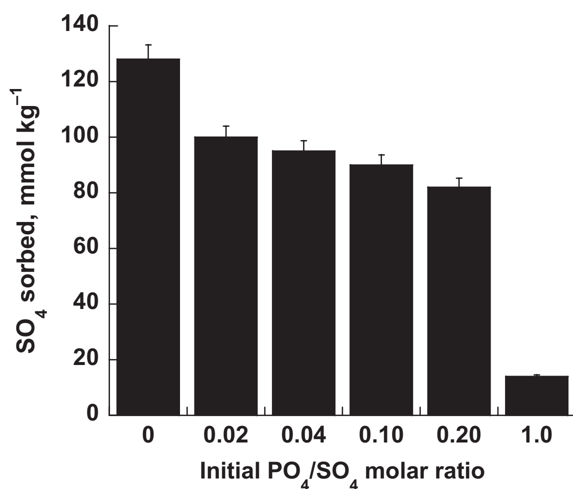


Figure 3.13 Sorption of sulfate (SO_4) on an Italian Andisol at pH 4.5 and in the presence of increasing concentration of phosphate (PO_4). Initial PO_4/SO_4 molar ratio ranging from 0 to 1. *(Redrawn from Pigna and Violante (2003)).*

arsenite, selenite; e.g. Geelhoed et al., 1997; Violante et al., 2008a; Zhu et al., 2011a, 2011b, 2011c) was larger at a lower pH particularly when added before the competing anion.

The extent of competition is related to sorption kinetics (Table 3.1). The effect of phosphate, citrate, or oxalate on the sorption of sulfate at pH 4.5 on an Andisol up to 13 days was studied by Jara et al. (2006). When added alone (100% surface coverage), sulfate sorption was fast in the first 2 days of reaction and then slowed down for a further 13 days. In the presence of equimolar amounts of phosphate (20% surface coverage), oxalate, or citrate (~50–60% of surface coverage), the sorption of sulfate was lower than that in the absence of foreign ligands even after many days of reaction. The efficiency of phosphate, oxalate, or citrate in preventing sulfate sorption after 48 h of reaction was 40, 42, and 61%, respectively, but after 15 days, it decreased to 27, 12, and 38%, respectively. Citrate prevented sulfate sorption more than the other ligands did, probably because of the high increase of the negative charge of the sorbent in citrate systems. Further, because the surface coverage of citrate was greater than that of phosphate, the competition with sulfate for common sorption sites was greater for the organic ligands than for phosphate.

Some researchers (Evans, 1991; Wolt et al., 1992) claimed that *adsorption* was not the only retention mechanism in sulfur-retentive soils. Formation of aluminum hydroxysulfate precipitates, including alunite [$KAl_3(OH)_6(SO_4)_2$] and basaluminate [$Al_4(OH)_{10}SO_4$], in acid soils containing sulfate or receiving sulfate has been widely reported (Sumner, 1993). Precipitation of hydroxy Al sulfates can be promoted by applying gypsum as an ameliorant for acid subsoils (Sumner, 1993).

Data reported in the literature on the removal of sulfate from clay minerals or soils are conflicting. Some scientists reported that very large percentages or all the sulfate sorbed were replaced in their sample soils or clay minerals with phosphate solutions (Pasricha and Fox, 1993). Conversely, others found that extractions with KH_2PO_4 did not remove all the sulfate present in Latosols and Brown Forest soils (Haque and Walmsley, 1973; Violante et al., 1996; and references therein). According to Haque and Walmsley (1973), only sulfate sorbed on surface groups of hydrous oxides was desorbed, whereas sulfate that penetrated into some amorphous region of the crystal surface was retained. Violante et al. (1996) demonstrated that sulfate was only partly removed from a synthetic aluminum hydroxysulfate, even after repeated washing with phosphate at pH 6.0, clearly because part of sulfate coprecipitated with Al was present in the network of the Al

precipitation product. Recently, sorption of arsenite and arsenate (which is an analog of phosphate) onto schwertmannite (a poorly crystalline Fe(III)-oxyhydroxysulfate mineral, which forms as primary product of Fe(II) oxidation and hydrolysis from acid sulfate waters), caused a significant removal of sulfate without degradation of the mineral structure, as ascertained by X-ray diffraction and Raman spectroscopy (Burton et al., 2009). These authors concluded that arsenic sorption occurred via incorporation into the structure of schwermannite rather than via complexation onto the surfaces of the mineral with an arsenate/sulfate exchange coefficient of 0.56–0.63 at pH 3.5–5.5 and of 0.22 at pH 9.0.

Sulfate and selenate poorly inhibited molybdate sorption onto an Al-oxide (Wu et al., 2001) as well as arsenate and arsenite on ferrihydrite but were much more effective in preventing arsenite on an Al-oxide (Zhu et al., 2011a, 2011b, 2011c). The influence of sulfate on arsenate, molybdate, and selenite was found to be insignificant on natural soils and Fe-oxides, but for quite high concentrations of sulfate (Jordan et al., 2009; references therein). However, sulfate strongly prevented arsenite sorption on ferrihydrite particularly at $pH < 7.0$, with the largest reduction at the lowest pH (3.0–5.0; Jain and Loeppert, 2000).

The charge distribution multisite complexation (CD-Music) model was used to predict the sorption between molybdate with sulfate, phosphate, silicate, and tungstate (Xu et al., 2006) on a goethite. Phosphate and tungstate were the strongest competitors of molybdate, whereas sulfate and silicate seemed to be the weakest. The model showed that deprotonated monodentate complexes were the dominant complexes.

Sulfate sorption in soils is negatively correlated with organic matter content, because organic ligands with high affinity for Al- and Fe-oxides may block sorption sites of variable charge sorbents and reduce their surface charge. Inskeep (1989) showed that humic, tannic, citric, gallic, and oxalic acids prevented sulfate sorption on kaolinite and Fe-oxides. Later, Martinez et al. (1998) studied the effect of 13 LMMOLs (eight aliphatic and five aromatic) on the sorption of sulfate on two variable charge soils. These soils, containing large amounts of citrate-dithionite-bicarbonate (CDB)-extractable Al and Fe, sorbed significant amounts of sulfate at pH 4.0 and 5.0. All the aliphatic acids, but cis-aconitic acid, prevented the sorption of sulfate, at both pH 4.0 and 5.0, with oxalic, tartaric, and citric acid, showing the greatest effect. Conversely, the aromatic acids and cis-aconitic acid usually increased sulfate sorption under certain conditions, probably because once sorbed these acids promoted the protonation of surface hydroxyl groups,

thus creating a more positively charged surfaces. The sorption of sulfate on natural soils was found to increase also in the presence of nitrate, chloride, perchlorite, carbonate, and monocarboxylic acids (acetic and formic acids) under certain conditions (Curtin and Syers, 1990). Wijnja and Schulthess (2000a, 2000b, 2002) studied the effect of carbonate and selected organic ligands on the sorption of sulfate and selenate onto an Al-oxide and goethite. Carbonate enhanced the sorption of these anions between pH 6.0 and 8.0 and at low CO_2 concentration (up to 0.3 mmol L^{-1}; Fig. 3.14A), but the promotive effect decreased by increasing CO_3 sorption (Fig. 3.14B). Acetate and formate showed a similar behavior, whereas oxalate and citrate always prevented sulfate and selenate sorption. According to these authors, under certain conditions, CO_3, acetate, and formate may promote the sorption of other oxyanions (mainly those with moderate affinity for the surfaces of the sorbents) by forming extra reactive protonated sites that exist with the sorbed CO_3 according to these reactions:



$$\text{Me[Fe or Al]OH}^{0.5+} + \text{HCO}_3^- = \text{Me[Fe or Al]OCOO}^{1.5-} + [\text{OH}^- + \text{H}^+]$$



$$\text{Me[Fe or Al]OH}^{0.5-} + \text{H}^+ = \text{Me[Fe or Al]OH}^{0.5-+}$$

where protons sorb concurrently with the sorption of CO_3 on adjacent or nearby sites, creating additional complexation sites.

Effects of carbonate on other anions (arsenate and arsenite) with a high affinity for Fe-oxides as a function of pH were studied by Arai et al. (2004) by using EXAFS and surface complexation modeling and Stachowicz et al. (2007) by using a charge distribution model. The former authors found suppression and enhancement of arsenate binding, respectively, at high or low CO_2 pressure, whereas the latter authors evidenced that dissolved bicarbonate acts as a competitor for both arsenate and arsenite and claimed that Arai et al. (2004), in contrast to the conditions in groundwater systems, used a very high arsenate concentration and a low CO_2 pressure.

Competition in sorption between phosphate with inorganic and organic anions has received great attention, as reviewed by Violante and Gianfreda (2000) and Violante et al. (2005, 2008a, 2008b). In the last decade, the competitive sorption of phosphate with arsenite and arsenate has been particularly studied (as reported below), arsenic an element being of great concern for human health.

Among the first studies on the influence of organic ligands on phosphate sorption are those of Johnston (1959) and Swenson et al. (1949).

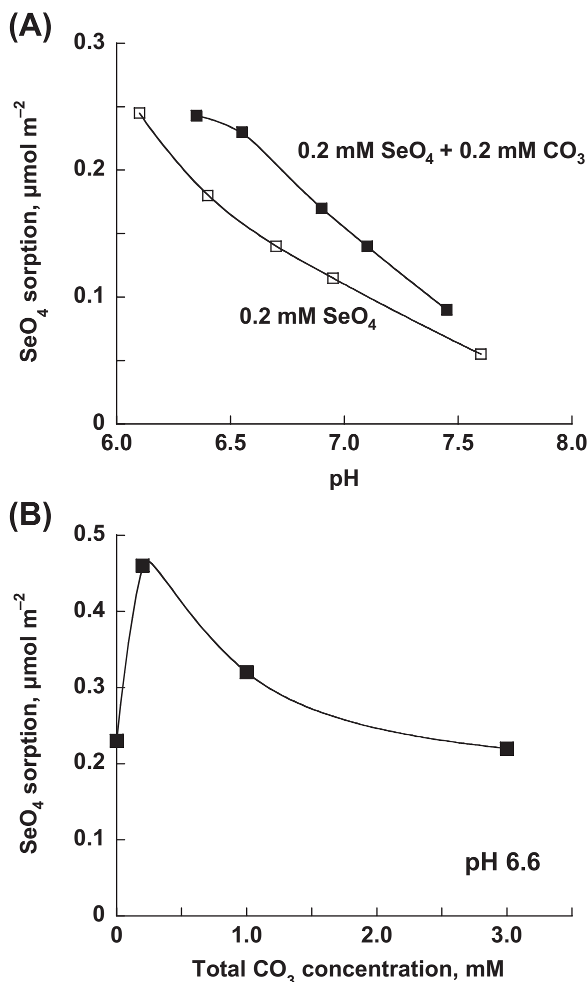


Figure 3.14 (A) Sorption of selenate (SeO_4) in the absence and presence of carbonate (CO_3) at indicated initial concentrations; (B) sorption of SeO_4 at pH 6.6 as a function of the total CO_3 concentration. Similar behavior for sulfate sorption. *(Redrawn from Wijnja and Schulthess (2002)).*

Many studies indicated that the most commonly occurring organic acids in soil, tri and dicarboxylic acids, were effective in reducing phosphate sorption, whereas monocarboxylic acids had little effect on phosphate fixation. Nagarajah et al. (1970) found that competitive capacity of carboxylic acids in inhibiting phosphate sorption on kaolinite, gibbsite, and goethite was in the order citrate > oxalate > malonate > tartrate > acetate. Phosphate sorption on goethite was not significantly influenced by the presence of

phthalate even if phthalate concentration was fivefold higher than that of phosphate (Nilsson et al., 1996). In the presence of citrate, phosphate sorption was affected at pH < 7.0 (mainly at pH 5.0), whereas citrate sorption decreased strongly over a wide pH range in the presence of phosphate (Geelhoed et al., 1998).

Competition in sorption among phosphate, tartrate, and oxalate, in both binary and ternary systems on OH–Al-montmorillonite complexes was studied by He et al. (1999). The sorption capacity of phosphate was much greater than that of oxalate and tartrate. Adding equimolar amounts of oxalate and tartrate resulted in an oxalate sorbed/tartrate sorbed molar ratio of ~1. Anyway, in the presence of phosphate the oxalate/tartrate molar ratios were <1 and decreased with increasing phosphate concentration, indicating that tartrate competed with phosphate more than oxalate for the sites common to all the ligands. Further, tartrate and oxalate added as a mixture were much more effective in inhibiting phosphate sorption than was tartrate or oxalate alone, probably because more sites with high affinity for the organic ligands were occupied by tartrate and oxalate than by tartrate alone.

The reduction of phosphate sorption in the presence of competing ligands was mainly attributed to competition for sorption sites and change in charge of the sorbents due to organic ligands fixation (as discussed before, Section 5.1). However, according to Lindegren and Persson (2009), the competition for goethite surface sites between carboxylic acids and phosphate is not due to a ligand–exchange reaction between *inner-sphere* surface complexes. Indeed, they found that polycarboxylic acids with high densities of functional groups, capable of multiple H-bonding interactions, are required to compete and desorb surface complexes of phosphate. These authors studied the competition in sorption between phosphate and oxalate, citrate, 1,2,3,4 butanetetracarboxylic acid (BTCA), mellitate, and FA at pH from 3 to 9 by batch experiments and ATR-FTIR spectroscopy (Fig. 3.15). The efficiency of these organic ligands follows the sequence oxalate < citrate < BTCA ~ FA < mellitate. Later, the same authors studied the competitive sorption between phosphate and seven benzene carboxylic acid containing increasing carboxylic groups. Only the acids with three or more carboxylate groups (BTCA, mellitate and FA) were able to partly compete with phosphate (Lindegren and Persson, 2010). Their findings evidenced that the most efficient were the ligands partially protonated and then the importance of both

H-accepting carboxylate groups and H-donating carboxylic acid groups for the competitive effect (Fig. 3.15).

Many research studies have been carried out on the influence of HA and FA on the sorption of phosphate, but limited conclusive evidence exists. Some studies showed that humic substances substantially decrease phosphate sorption (e.g. Bhatti et al., 1998; Sibanda and Young, 1986), whereas others concluded that they have a poor influence on phosphate sorption on variable charge minerals and soils (Borggaard et al., 2005). Antelo et al. (2007) demonstrated that phosphate and HA competed for sorption on goethite. The presence of HA decreased phosphate sorption and vice versa, but the magnitude of competition was affected by pH. Precisely, a greater reduction in phosphate sorption occurred at low pH

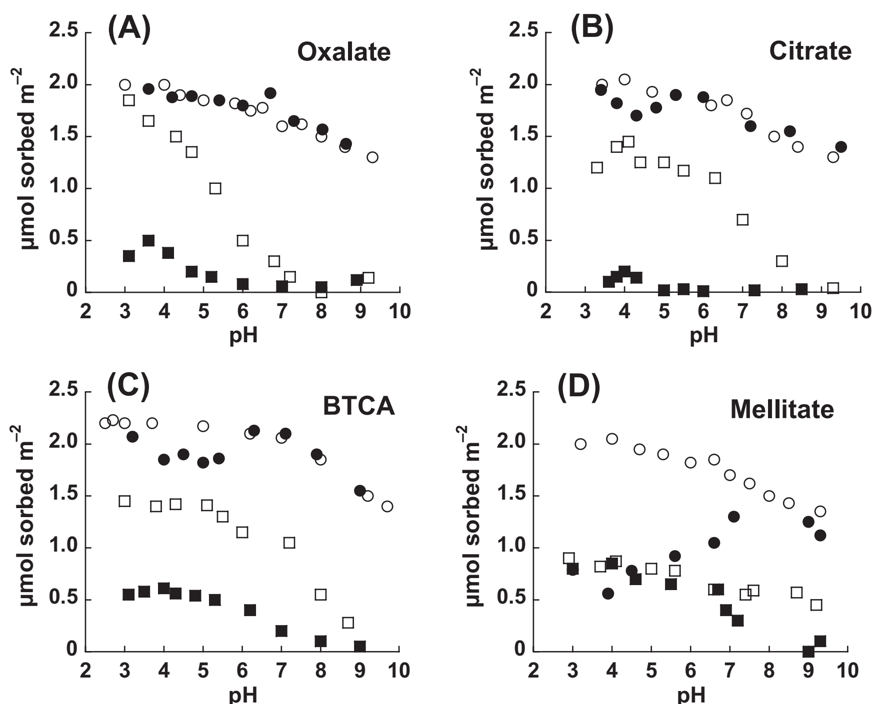


Figure 3.15 Sorption of organic acid [(A) oxalate, (B) Citrate, (C) BTCA and (D) Mellitate] and phosphate as a function of pH with a reaction time of 24 h □ denotes sorption of the organic ligand at the single ligand–goethite system and ■ in the competitive organic acid–phosphate–goethite system. ○ denotes sorption of phosphate in the phosphate–goethite system and ● in the competitive organic acid–phosphate–goethite system. All ligands are simultaneously added in a concentration of 2.7 ± 0.3 mmol m^{-1} of goethite. *(Redrawn from Lindegren and Persson (2010)).*

(pH 4.5), whereas a greater decrease in HA sorption occurred at pH 7.0. According to these authors, both phosphate and HA may compete for common surface sites, and also electrostatics play an important role in the competition. Further, equilibration was reached more quickly when the competitors were added as a mixture than when one of them was added before the other.

Recently, Weng et al. (2008) gave a valuable explanation for the sorption of phosphate in the presence of HA or FA on goethite. The sorption of phosphate in the presence of HA or FA was well predicted with the ligand and charge distribution. According to the model, the nature of interactions between HA or FA with phosphate is mainly electrostatic. These authors found that HA was strongly bound on the goethite surfaces but did not prevent the sorption of phosphate so effectively as FA did (by a factor up to 100 at low pH) despite FA being less strongly bound to the Fe-oxide than HA was. Further, in the presence of phosphate, FA sorption decreased substantially over the entire pH range (3.0–7.0), whereas HA sorption was less inhibited particularly at low pH. These effects have been attributed to the difference in the spatial distribution of HA and FA in the goethite–water interface, the latter being sorbed closer to the oxide surface, so that the electrostatic interactions between FA nanoparticles and phosphate are much stronger than for HA nanoparticles (Fig. 3.16A). In conclusion, the greater effect of FA to prevent phosphate sorption was attributed by these authors to a combination of a larger number of nanoparticles sorbed (although the amounts in terms of mass is lower) and a shorter distance to the surface (Fig. 3.16B). The available surface sites were reduced for the formation of *inner-sphere* complexes of HA or FA on the surfaces of goethite, favoring the formation of monodentate over bidentate phosphate species sorbed, but model calculation showed that the effects of electrostatic potential is of much greater importance.

The effect of agrochemicals on phosphate sorption also received attention. Particularly interesting were the studies on the competition in sorption between phosphate and glyphosate (Gimsing et al., 2007; and references therein). More glyphosate (nearly double) than phosphate was sorbed on tropical soils when these ligands were added alone; the former formed monodentate complexes, whereas the latter bidentate complexes. However, phosphate was more strongly bonded than glyphosate was. In competitive experiments, initial addition of glyphosate resulted mainly in additive sorption of glyphosate and phosphate,

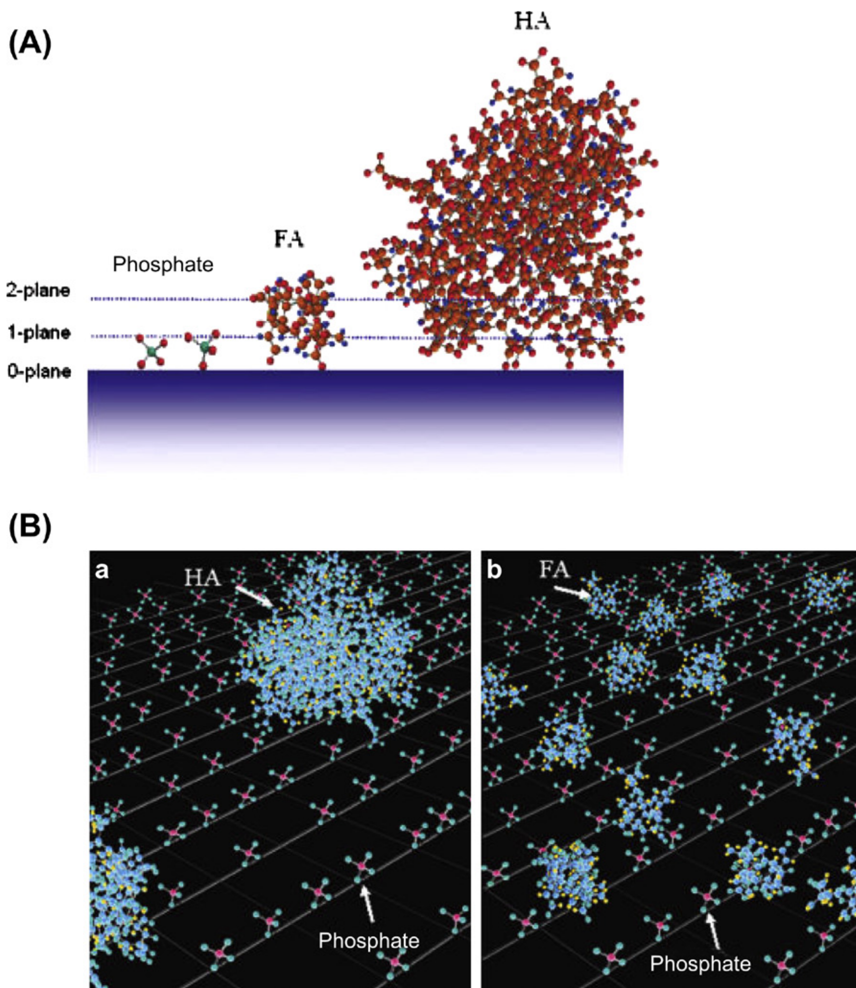


Figure 3.16 (A) Schematic representation of phosphate, FA, and HA at the goethite surface in which the extended Stern model is used to describe the structure of the electric double layer; (B) schematic representation of goethite surface (about 100 nm^2) covered with phosphate and HA (a), or phosphate and FA (b). For color version of this figure, the reader is referred to the online version of this book. *(Reprinted with permission from Weng et al., (2008))*.

phosphate being able to remove small amounts of glyphosate. Vice versa, presorbed phosphate increased the negative charge on the soil surfaces and suppressed glyphosate sorption. Additive and competitive sorption seem to dominate the sorption of glyphosate and phosphate on variable charge soils.

5.3. Competitive Sorption Involving other Anions

The effect of *silicic acid* on the sorption of other anions onto soil components has received relatively scarce attention, despite silicic acid being a ligand, which is ubiquitous in natural environments. The concentrations of silicic acid in soils and natural waters range from 0.04 to 0.380 mmol L^{-1} with some as high as 0.814 mmol L^{-1}. The ability of silicate to compete with other anions is highly pH dependent because silicic acid is weakly dissociated at pH < 9.7, which limits its effectiveness to compete with ligands in the pH range of most soils. The presence of silicic acid decreased the rate and total quantity of chromate sorption onto goethite mainly at pH > 4 (Garman et al., 2004). The inhibition of chromate sorption ranged from 0 to 83.3 and 3.1–63.9% for 0.05 and 0.1 mmol L^{-1} chromate, respectively. The molar ratio of chromate sorbed to silicic acid desorbed was >1, demonstrating the presence of excess surface sites for oxyanions sorption.

The sorption of silicic acid (added 60 h before arsenic) also decreased the rate and the total amount of arsenite and arsenate sorbed (Waltham and Eick, 2002). According to Swedlund and Webster (1999), silicic acid is sorbed on ferrihydrite as a monomer when Si/Fe molar ratio is <0.1, whereas at greater Si/Fe molar ratios, polymerization of silicic acid on the surface of the Fe-oxide is increasingly important (Fig. 3.17a–c). By studying the competition of silicic acid and arsenite or arsenate, these authors found that diffuse layer model well predicted the effect of silicic acid on arsenite and arsenate sorption even at Si/Fe = 1.8. Later, Luxton et al. (2006, 2008) and Swedlund and Webster (1999) demonstrated that silicate polymers did not significantly affect arsenite sorption as compared to monomers (a reduction of arsenite up to 15%). The latter authors also ascertained that polymerized silica is competitively displaced by arsenite but not released into solution (Fig. 3.17). Lee and Kim (2007) found that silicate particularly when added to soil before phosphate greatly reduced phosphate sorption at pH > 7.0. The effect of phosphate in reducing silicic acid was relatively small at pH 5.0, but no effect was evidenced at pH 9.0. According to Meng et al. (2000), arsenite sorption was significantly decreased in the presence of silicate when Si concentration was >1 mg ml^{-1} and at pH > 5.0, whereas arsenate was only moderately removed.

Jordan et al. (2009) studied the competition in sorption between *selenite* and silicic acid. They found that the sorption of selenite decreased in a sharp pH range, while sorption of silicic acid occurred over a wide pH range.

The experimental observation was well fitted by surface complexation model. Balistrieri and Chao (1987) suggested that for a given anion concentration ratio, the competition sequence with selenite on goethite is phosphate > silicate > citrate > molybdate > bicarbonate/carbonate > oxalate > fluoride > sulfate. The same authors (Balistrieri and Chao, 1990) found that the competition sequence of selected anions with respect to selenite sorption at pH 7.0 was phosphate > silicate > molybdate > fluoride > sulfate on noncrystalline Fe oxyhydroxides and molybdate ≥ phosphate > silicate > fluoride > sulfate on manganese oxides. Selenate poorly competed with the anions under investigation and did not sorb on manganese dioxide. Later, Dynes and Huang (1997) showed that the ability of 12 LMMOLs to inhibit selenite sorption on poorly

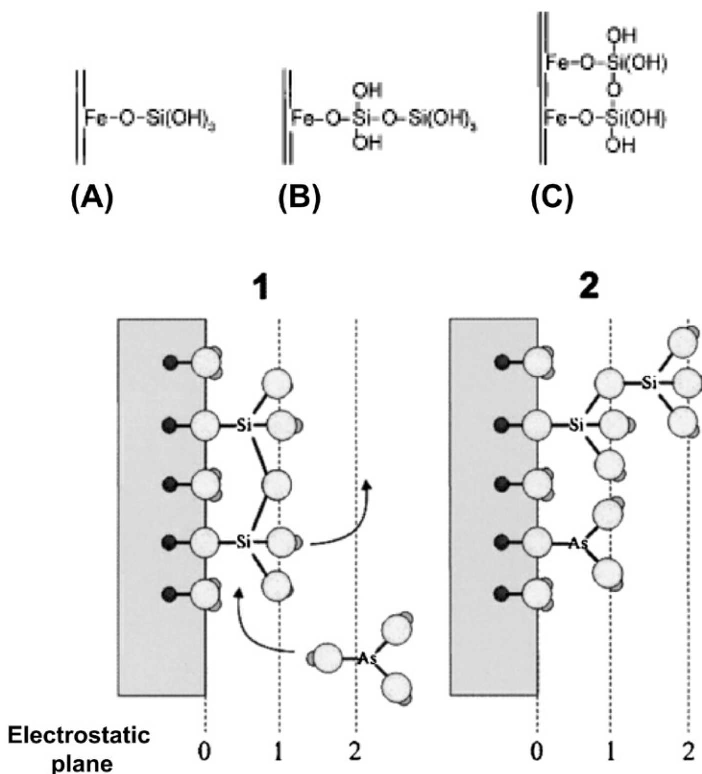


Figure 3.17 (A) Schematic bonding for H_4SiO_4 sorption onto ferrihydrite. (a) Sorption of H_4SiO_4 monomer, (b) polymerization, and (c) siloxane linkage between adsorbed H_4SiO_4 monomers *(from Swedlund and Webster (1999) with permission)*. (B) Schematic representation of depicting polymerized silica on the goethite surface being displaced by arsenite [As_2O_3] but not released into the bulk solution. *(Reprinted with permission from Swedlund and Webster (1999))*.

crystalline Al-hydroxides was oxalate > malate > citrate > succinate > glycolate > aspartate > salicylate > *p*-hydroxybenzoate > glycine = formate = acetate. Generally, the larger the stability constant of the Al-organic solution complexes (K_{Al-L}), the more effective the organic acid was in competing with selenite for the sorption sites of the Al-hydroxides. However, some of the organic acids competed less successfully than was expected based on their K_{Al-L} values. This was attributed to the stereochemical and electrostatic effects originating from both the surface of the Al-hydroxides and the organic acids, which lowered the ability of some organic acids to compete with selenite for the sorption sites of the Al-hydroxides.

Competition studies among selenite, arsenate, and vanadate onto alumina showed that the sorption affinities of these three elements followed the sequence arsenate > vanadate > selenite (Su et al., 2010). A speciation-based model was developed to quantify the competitive sorption behavior of these elements.

Studies on the competition in sorption between arsenic (mainly *arsenate* and *arsenite*) and inorganic or organic anions have received great attention in the past decades, because health problems related to its presence in natural environments have been worldwide recognized. Phosphate is added to soil as a plant nutrient and may then affect the mobility of arsenic (Frankenberger, 2002; Smedley and Kinniburgh, 2002; Violante et al., 2005, 2008a, 2008b). Although arsenate is chemically similar to phosphate, competition in sorption between phosphate and arsenate varies greatly on different soil minerals and soils characterized by different mineralogies and chemical properties (Frankenberger, 2002; Violante and Pigna, 2002; Violante et al., 2008a, 2008b, and references therein). Violante and Pigna (2002) evidenced that Mn, Fe, and Ti oxides and phyllosilicates particularly rich in Fe (nontronite, ferruginous smectites) were more effective in sorbing arsenate than phosphate, but more phosphate than arsenate was sorbed on noncrystalline Al precipitation products, gibbsite, boehmite, allophane, and kaolinite. Competitiveness between the anions also changed at different pH values (Table 3.2). The effect of phosphate to prevent arsenate sorption increased on increasing pH values. Jain and Loeppert (2000) found that phosphate strongly reduces the sorption of both arsenate and arsenite onto ferrihydrite, but the effect of phosphate on the sorption of arsenite (differently from arsenate) was greater at low pH. Arai and Sparks (2002) demonstrated that the longer the residence time (3 d–1 y), the greater the decrease in arsenate desorption by phosphate from a bayerite. The effect of anions concentration, residence time, surface coverage, and component sequence

Table 3.2 Sorption of Phosphate (PO_4) and Arsenate (AsO_4; mmol kg^{-1}) on Phyllosilicates, Variable Charge Minerals, and Soils at pH 4.0 and 7.0, When the Anions were Added Alone or as a Mixture (initial AsO_4/PO_4 molar ratio of 1.0)

	Anion added alone		**Anion added together**			**Anion added alone**		**Anion added together**		
Samples	**PO_4**	**AsO_4**	**PO_4**	**AsO_4**	***rf***	**PO_4**	**AsO_4**	**PO_4**	**AsO_4**	***rf***
	pH 4.0					**pH 7.0**				
Smectite ferr.	21.3	22.6	14.5	18.7	*1.29*	19.4	18.9	16.8	15.8	*0.93*
Montmorillonite	14.7	12.5	11.1	9.2	*0.82*	11.4	8.5	8.7	7.2	*0.83*
Vermiculite	13.1	12.9	12.4	12.1	*0.98*	8.0	7.5	7.3	6.6	*0.90*
Kaolinite	20.8	10.0	19.6	6.4	*0.32*	12.7	8.1	11.6	5.3	*0.45*
Birnessite	28.6	29.9	16.0	27.7	*1.73*	15.8	17.3	8.2	17.3	*2.10*
Goethite	160.0	193.0	79.0	92.0	*1.16*	136.0	152.0	73	72	*1.00*
Allophane	1315.0	1218.0	1300.0	630.0	*0.48*	188.0	113.0	200.0	10.0	*0.05*
Gibbsite	318.0	300.0	277.0	130.0	*0.45*	225.0	151.0	161.0	60.0	*0.37*
Oxisol	72.6	54.1	57.7	33.3	*0.58*	60.8	45.0	49.6	27.2	*0.55*
Andisol	410.0	284.0	331.0	124.0	*0.37*	269.0	179.0	209.0	68.5	*0.33*

rf stands for sorbed AsO_4/PO_4 molar ratio.
Modified from Violante and Pigna (2002).

addition on the sorption/desorption of phosphate and arsenate has been reviewed by Frankenberger (2002) and Violante et al. (2005, 2008a, 2008b).

Coprecipitation of arsenic (as well as other metal[loid]s) with Al or Fe in soil environments occurs easily. Arsenic accumulation in iron plaques (mainly poorly crystalline precipitates) of plant roots has been studied (Violante et al., 2012; and references therein). Violante et al. (2006, 2007, 2009a, 2009b) demonstrated that less arsenate was replaced by phosphate from arsenate iron and/or aluminum coprecipitates than from previously formed iron and/or aluminum oxides on which arsenate was sorbed (Pigna et al., 2006). Low amounts of arsenate coprecipitated with aluminum and/or iron oxides at pH 7.0 were removed by phosphate (5%–25%), attributed to the formation of strong *inner-sphere* complexes, metal–arsenate precipitates and partial occlusion of arsenate into the coprecipitates. A comparison of the desorption of arsenate by phosphate from aluminum–arsenate, iron–arsenate, and iron–aluminum–arsenate coprecipitates evidenced that phosphate desorbed more arsenate from aluminum–arsenate, iron–aluminum–arsenate than iron–arsenate coprecipitates (in the order listed), because arsenate forms

stronger complexes with Fe than with Al. Greater amounts of arsenate were sorbed on Al–Mg or Fe–Mg–LDHs than on noncrystalline or poorly crystalline Al- or Fe-oxides (Caporale et al., 2011), but phosphate was able to replace large amounts of the metalloid from these sorbents reaching a percentage of arsenate removed up to 70% from Al–Mg–LDH and 63% from Fe–Mg–LDH.

Jain and Loeppert (2000) also studied the competition in sorption between arsenate and arsenite on ferrihydrite in the range of pH 3–10. When the concentrations of the two arsenic species were <2 mol kg^{-1} each, the influence of arsenate in preventing arsenite sorption was more evident than vice versa. On the contrary, when the concentration of arsenate and arsenite was 3.5 mol kg^{-1} each, arsenate did not affect the sorption of arsenite, whereas arsenite strongly prevented arsenate sorption, probably due to surface polymerization of sorbed arsenite at high concentrations. However, their polymerization hypothesis needs to be well demonstrated.

In the last decade, the effect of LMMOLs on arsenic sorption has received attention (Grafe et al., 2001;Violante et al., 2005, 2008; Zhu et al., 2011b).Violante et al. (2005) found that the efficiency of selected LMMOLs in inhibiting arsenate sorption on an Andisol was citric acid > malic acid > OX >> succinic. At organic ligand/arsenate molar ratio of 4, citric, malic, and OX reduced arsenate sorption of 55, 40, and 15%. This behavior may be due to the different affinities of the LMMOLs studied for Al. In fact, the citric-, malic-, and oxalic-Al complexes at stoichiometry of the complex Al-ligand 1:1 have stability constants of 7.9, 6.1, and 6.0, respectively. The efficiency of organic ligands in preventing arsenate sorption was affected by the nature and surface properties of the sorbents. Malic acid at pH 4.0 poorly inhibited arsenate sorption on pyrolusite and goethite even at a malic acid/ligand molar ratio of 5, whereas it strongly prevented arsenate sorption on gibbsite and an Andisol sample containing 40% of allophane, attributed, as reported before, to the greater affinity of arsenate for Mn and Fe than for Al (Violante and Pigna, 2002, Table 3.2). Grafe et al. (2001) found that arsenate sorption on goethite decreased in the presence of HA > FA > citric acid, while arsenite sorption was decreased in the presence of citric acid > FA ≈ HA.

Recently, Zhu et al. (2011b) studied the effect of organic and inorganic ligands on the sorption of arsenite and arsenate on ferrihydrite. The efficiency of the anions studied in preventing arsenite and arsenate sorption was as follows: selenate ≈ sulfate < oxalate < malate ≈ tartrate < citrate

< selenite << phosphate. The efficiency of most of the competing anions in inhibiting the sorption of the metalloid decreased by increasing the pH, but phosphate whose efficiency increased at higher pH values. In acidic environments, all the ligands prevented the sorption of arsenite more than that of arsenate, but in alkaline systems, arsenite and arsenate seemed to be retained with the same strength. Caporale et al. (2011) demonstrated that the capacity of selected ligands in preventing arsenate sorption onto AlMg– or FeMg–LDH followed the sequence nitrate < nitrite ≈ sulfate < tartrate < oxalate < selenite << phosphate.

Some investigations have demonstrated that FA can reduce arsenate sorption in a range of minerals and soils (Bowell, 1994; Grafe et al., 2001), but no competition in sorption was observed for ferrihydrite (Grafe et al., 2002). The exact mechanisms causing arsenic removal in the presence of FA remain unclear. The microbial and chemical reduction of Fe-oxides has been also suggested as possible mechanisms for releasing sorbed arsenic (Jones et al., 2000; Nickson et al., 2000). Simeoni et al. (2003) demonstrated that arsenate sorption decreased on ferrihydrite and gibbsite by increasing FA concentrations (particularly at pH 4.0), and the release of previously sorbed arsenate from ferrihydrite was not caused in their experiments by the reduction of the Fe-oxide by FA. These authors demonstrated that the order of the addition of arsenate and FA affected the sorption of the metalloid, whereas Grafe et al. (2002) found no change in arsenate sorption. These differences were attribute to a greater surface coverage of arsenate onto the surfaces of ferrihydrite in the experiments of Simeoni et al. (2003). According to these authors, adding FA (~1.5 nm in diameter) to the mineral before arsenate blocks sites contained in pores, approaching the size of FA and which would be available for arsenate. Vice versa, FA cannot replace arsenate present within the porous structure of ferrihydrite, as reported in Fig. 3.18.

The fixation of oxyanions on organic matter as affected by the presence of metal cations (mainly iron and aluminum) is a subject of study of great importance. Many binding mechanisms have been proposed by Mikutta and Kretzschmar (2011) for arsenate and arsenite sorption to organic matter, which include the formation of (i) *outer-sphere* complexes with protonated amino groups, (ii) covalent bonds with carboxylate or phenolate groups, and (iii) the formation of ternary complexes with polyvalent cations (Fe, Al) forming a bridge between arsenic anions and organic matter. These authors showed spectroscopic evidence for the formation of ternary complex between arsenate, ferric ions, and humic substances, with

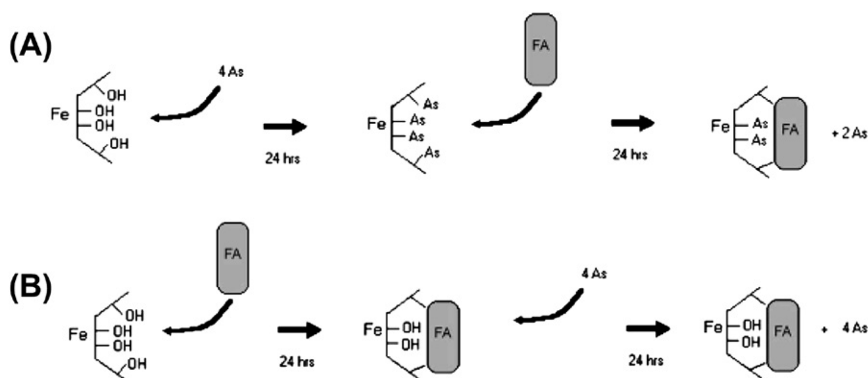


Figure 3.18 A schematic diagram of the effect of FA on arsenate on a rough or porous ferric hydroxide structure: (a) addition of arsenate (As) at 0 h, FA 24 h; (b) addition of FA at 0 h and arsenate at 24 h. In scheme b, the blockage of available sites reduces the sorption of arsenate on ferric hydroxide surfaces. *(Reprinted with permission from Simeoni et al. (2003)).*

at least 70% of arsenate bound to Fe(III)–HA complexes via *inner-sphere* complexation.

6. CONCLUSIONS

In soils and related environments, inorganic and organic ions, neutral substances, different minerals, humic substances, and organisms are present together and interact with one another in a multitude of ways that are difficult to predict. Even the processes that affect the *simple* competition in sorption of different ions onto the surfaces of a single soil component are very complex and not easy to understand. In the past decades, to know the factors that affect the mobility of cations and anions in soil environments, macroscopic and spectroscopic (ESR, XAS, EXAFS, XANES) studies, dealing with the mutual influence of different ions in competing for sorption sites of soil components (mainly on minerals and in ternary systems), have significantly increased.

In this chapter, we provided the current state of knowledge on the factors that influence the mutual interactions of cations and/or anions at solid/water interface. The mutual influence of different ions in competing for sorption sites of a sorbent is mainly caused by site competition and/or electrostatic effects. Usually, for ions with the same charge, these two effects have a negative mutual influence on the sorption, whereas for ions with an opposite charge, the electrostatic effect have a positive mutual influence on

the sorption, but for competitive sorption of cations and anions, a combination exists between synergy and competition.

The competition between two or more cations and/or anions is affected by pH, the nature and concentrations of the competing ions, oxidative state, surface properties of the sorbents, sequence of addition of the interfering components, and residence time.

The sorption of metal cations on soil components is strongly affected by the presence and concentration of organic and inorganic ligands [nutrients, HAs and FAs, and LMMOLs (e.g. root exudates)] due to complexation reactions of polyvalent cations with chelating ligands. The presence of a foreign anion usually decreases the sorption of cations onto negatively charged sorbents by forming negatively charged complexes, but certain siderophores and phytosiderophores may enhance the fixation of polyvalent cations under certain conditions forming positively charged complexes. In contrast, the sorption of cations in the presence of inorganic and organic ligands onto the surfaces of variable charge minerals may be affected by several processes, which may occur simultaneously. Increased retention could be favored by the decrease of the positive charge of the mineral surfaces when a ligand is sorbed and to the formation of negatively charged complexes between a metal and a ligand, which have a higher affinity for the sorbent; vice versa, decreased retention could be due to the block of sorption sites by foreign ligands on the minerals and to the strong complexation of metal in solution in nonsorbing form as well as dissolution of the mineral in the presence of relatively high concentration of chelating organic ligands. Spectroscopic studies have permitted us to have important information on the conditions that favor the formation of sorbent–metal–ligand (type A) or sorbent–ligand–metal (type B) ternary complexes on different minerals as well as the formation of surface precipitates. Spectroscopic studies have also evidenced that competition between ions may alter the balance of *inner-* and *outer-sphere* complexes of a given sorbate (Fig. 3.5) and the distribution of monodentate, bidentate, or tridentate complexes of a metal cation on different faces of an oxide (Fig. 3.6).

Anions are easily sorbed on variable charge minerals. The presence of a competing anion usually decreases the sorption of another anion. Competition between anions is attributed to competition for the occupancy of sites available onto the surfaces of the sorbents and a change in electric potential of the surfaces due to sorption of anions. The influence of electric potential is particularly important when competition occurs between ligands with a great difference in affinity for the surfaces

of a sorbent (e.g. phosphate and sulfate). The extent of competition is also related to sorption kinetics. However, some ligands (carbonic acid, monocarboxylic and aromatic acids) may increase sorption of competing anions onto variable charge minerals by promoting the protonation of surface hydroxyl groups, thus creating more positively charged surfaces. In this chapter, we discussed the factors that affect the competition of different anions, such as, sulfate, phosphate, arsenate, arsenite, selenite, silicate, LMMOLs, FA, and HA.

Despite much information already being available, certainly in future there is a need for more basic research on interactions between different ions, complexing ligands, clay minerals, humic substances, microorganisms under different conditions to better understand the mechanisms of these interactions at the molecular and atomic level. To reach these goals, interdisciplinary research studies are also necessary. Anyway, the most fundamental and innovative research will also depend on the development and more extensive application of powerful spectroscopic techniques. At the moment, advanced modern techniques have some serious limitations (e.g. limited sensitivity) and are not easily accessible to many scientists (Jackson, 1998; Sparks and Ginder-Vogel, 2012). However, as reported by Sparks and Ginder-Vogel (2012), these problems can begin to be solved by advances in both user support and new advanced instrumentations, as, for example, third-generation synchrotron light sources, which will allow for the analysis of lower concentration elements.

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CHAPTER FOUR

Insights from the Soybean (*Glycine max* and *Glycine soja*) Genome: Past, Present, and Future

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Contents

Abstract

The complete assembly of the soybean genome sequence was a paradigm-shifting event for legume genomicists and offered a promising new resource for breeders and geneticists. Subsequent and ongoing resequencing of additional soybean accessions and wild relatives is building a comprehensive infrastructure for understanding soybean gene content and germplasm diversity. In this chapter, we first revisit the major events in soybean genomics that preceded and led to the soybean-sequencing project. Next, we delve into the important and unique features of the soybean genome that have been revealed through recent sequencing and resequencing efforts. We highlight the immediate impacts of the genome sequence, including the integration of the soybean genetic and physical maps and how the

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genome sequence has accelerated gene cloning. Last, we address ongoing and new projects that are leveraging the genome sequence to address previously inaccessible questions, and providing new genetic resources. The purpose of this chapter is to give an insight into the ways that the soybean genome has enabled the research community to address standing issues in both fundamental and applied areas.

1. INTRODUCTION

The assembly of the soybean (*Glycine max*) genome sequence was a landmark event in the history of legume biology (Schmutz et al., 2010). The public release of the cultivar Williams 82 genome sequence was almost immediately followed by studies in which additional soybean accessions and several wild *Glycine soja* accessions were resequenced and compared with the Williams 82 reference (Kim et al., 2010; Lam et al., 2010). The reference genome sequence has become a primary tool for investigating soybean diversity and gene function. As evidence of this, the original article describing the genome sequence that was published in January 2010 has already been cited over 400 times in the primary literature—a number that will likely multiply 10-fold in the coming years. However, for readers not intimately involved in genomics, the soybean genome sequence and resequencing datasets may seem to be of limited value, just a colossal mass of data present on a server.

The primary purpose of this review is to highlight the literature that has advanced our understanding of the soybean genome structure and function. The second purpose is to explore the ways in which the soybean genome is enabling advances in breeding and improvement. It is not uncommon for growers and commodity board members to enquire about the long-term plans for soybean genomics and crop improvement. We hope that this chapter will help merge the gap between the stupendous masses of data being accumulated and the utility of such data for advancing the state of soybean research, both fundamental and applied.

2. THE PREGENOMIC ERA

The pregenomic era can be roughly defined as the timeframe before the completion of the soybean genome sequence. While the vast majority of history of soybean is outside the scope of this chapter, we will provide some context of the story that is particularly relevant to modern-day geneticists and genomicists.

2.1. Phenotypic Diversity, Domestication, and Breeding

The cultivated soybean [*G. max* (L.) Merr.] was domesticated from the wild soybean (*G. soja* Seib. et Zucc.), according to the Chinese scholar Gai, (1997). The Chinese literature during the Shang dynasty from 1500 to 1100 BC documents the use of soybean in that timeframe (Qiu et al., 1999), and there are anecdotal references to its use in earlier time periods (Guo, 1993), leading Gai (1997) to state that domestication occurred approximately 5,000 years ago. However, a US authority on this subject discounts the anecdotal information (Hymowitz, 2004) and asserts that domestication occurred about 3500 years ago in the eastern half of north China, either during the Shang dynasty or shortly before. After domestication, soybean domesticates were subsequently introduced to other southeastern Asian countries via trading routes. Interestingly, the introduction of soybean into Japan may not have occurred until about 2000 years ago (Li and Nelson, 2001), and unlike other countries, the Japanese soon made soybean oil and protein an essential inclusion in their daily diets.

The foregoing suggested domestication time frames may need updating in light of recent archeological evidence. Lee et al. (2011) conducted a comprehensive examination of the seed size of 949 charred soybean specimens collected from 22 sites and, based on that analysis, suggested that soybean may be as old as 9000–8600 calibrated years before the present (cal BP) in northern China and about 7000 cal BP in Japan. Direct accelerator mass spectrometry radiocarbon dates on charred large soybean seeds suggested that selection for seed of a domestication seed size had already occurred in Japan by 5000 cal BP, and in Korea by 3000 cal BP. Moreover, the archaeological record supports a hypothesis that soybean was domesticated in not one but probably several locations in East Asia.

Soybean was likely not introduced into the USA until 1765 (Hymowitz and Harlan, 1983), and then only for the purpose of producing soy sauce, but soybean was later used as a forage crop until about 1915, when soybean seed was first crushed in the USA to create soybean cooking oil (Mounts et al., 1987). Soon thereafter, soybean transitioned from a forage crop into an important seed crop, and by the 1930s, breeding programs had been established at United States Department of Agriculture (USDA) research locations and State Agricultural Experiment Stations in the US soybean growing areas (Bernard et al., 1988). Soybean is currently the second most valuable crop after maize grown in the USA, and the two crops are commonly grown in rotation in the northern USA.

The wild soybean, *G. soja*, is sexually compatible with the domesticated soybean and still grows as a wild plant in the East Asian countries of China, Japan, Korea, Taiwan, and Russia (Hymowitz, 2004). Although *G. soja* is considered to be a primary gene pool for domesticated soybean, it has rarely been used for that purpose by soybean breeders. The Asian *G. max* landraces are used more often by breeders as sources of genes for various traits. The more distantly related perennial *Glycine* species are indigenous to Australia and are categorized as being a tertiary or quaternary gene pool for soybean (Chung and Singh, 2008). These perennial species are sexually incompatible with *G. max*, and have no footprint in the history of soybean breeding.

Cultivated soybean germplasm has long been recognized to have a relatively narrow genetic base (Carter et al., 2004), as compared with other crop species. The limitation is even more severe in the North American germplasm (Specht and Williams, 1984). Of the nearly 260 North American public cultivars released from 1947 to 1988, about 80 ancestors accounted for 99% of the parentage (Gizlice et al., 1994), though 90% of the parentage was attributable to just 26 of those ancestors. Carter et al. (2004) recently tabulated a list of the 21 ancestors that account for 87% of the proprietary cultivars released in North America from 1999 to 2001, but noted that the northern and southern US breeding pools were quite distinct in terms of pedigrees that trace back to 17 in-common ancestors. Despite this historical pedigree-based evidence of a lack of genetic diversity, a wide range of phenotypic variation is observable in the cultivated *G. max* germplasm. For example, soybeans have been adapted for a wide range of maturity zones, ranging from the indeterminate photoperiod insensitive cultivars of Canada to the determinate photoperiod sensitive cultivars grown in the equatorial tropics.

When soybean breeding efforts initiated in the 1930s (Hartwig, 1973), the focus was on evaluating plant introductions, purifying these, then releasing these as named cultivars. However, this method only exploited the genetic variability *between* such introductions (Specht and Williams, 1984). Soybean breeders subsequently moved on to create biparental matings between the (then) best introductions, thereby creating new genetic variability in the form of transgressive segregant extremes (tails) in the phenotypic distributions, with breeders selecting the positive tail segregants for release to soybean producers. Specht and Williams (1984) demonstrated that this change in breeding method resulted in a one-time quantum jump of 25% in soybean yield that, in effect, was equivalent to the advancement in maize yield that occurred when open-pollinated populations were replaced

with single-cross hybrids. The three-phrase cyclic process of biparental hybridization, selfing, and testing, wherein the best progeny identified at the end of a cycle serve as the parents for initiating the next cycle, remains the primary means today of generating soybean cultivar releases to producers. This cyclic process is, of course, now supplemented with the application of genomic tools and transgenic technologies. In fact, genetically modified soybeans resistant to the herbicide glyphosate became commercially available in 1996, and the cultivars containing this transgene now dominate the market in both North and South American soybean production systems.

2.2. Cytogenetics

Although it was known in 1934 that a haploid soybean cell has 20 chromosomes (Veatch, 1934), conventional karyotype analysis in soybean is difficult because of a large number of chromosomes per cell (20 pairs) that are small in size and morphology and similar to each other. Hadley and Hymowitz (1973) reviewed the (then) existent soybean cytogenetic literature, and stated rather succinctly "Cytological information concerning the soybean is meager." The authors did provide photographs of meiotic chromosomes in *G. max* and some of the perennial species. About a decade and a half later, Hymowitz and Singh (1987) discussed their cytotaxonomic research on the perennial soybean species. Palmer and Kilen (1987) reviewed the soybean cytological studies and found it still sparse, but did note some published reports that the Deoxyribonucleic acid (DNA) content per soybean haploid nucleus was 1.97 pg (as determined by reassociation kinetics), ranged from 1.84 to 2.61 pg (as ascertained via cytophotometry), and was lower in domesticated soybean versus the wild soybean. Hymowitz (2004) provided the most recent summary of soybean cytogenetics research, and noted that the application of biochemical and molecular tools had stimulated more phylogenetic research in soybeans since the last review. Several chromosomal aberrations, such as reciprocal translocations and inversions, have been identified and preserved. Despite the fact that the domesticated *G. max* and wild *G. soja* are diploidized ancient polyploids, aneuploids of the deficiency type have been difficult to detect (i.e. only two monosomics have been reported). In contrast, aneuploids of the addition type—primarily trisomics—can be detected and, in fact, 20 trisomic lines were created. Many but not all of the trisomic lines were used to align specific classical linkage groups (CLGs) with the chromosomes depicted in the ideogram of each of the soybean pachytene chromosomes that was presented in Hymowitz (2004). However, this trisomic mapping effort had not been completed when the soybean

genome was sequenced, so the numbering system in that ideogram does not entirely match the chromosome numbers that had to be chosen for use in the soybean-sequencing project (Hyten et al., 2010). Most cytogenetics research since 2004 has focused on the molecular features of the soybean chromosomes and will be discussed later in this review.

2.3. Classical and Molecular Marker Linkage Maps

The term "classical markers" has traditionally been used to designate genes inferable from the inheritance patterns of segregating phenotypic contrasts, wherein the latter are either directly observable by eye, or are readily scorable in a qualitative manner after application of an easily implemented assay (e.g. inoculation of plants with viral, bacterial, or fungal pathogens or specific races thereof). Published soybean linkage maps involving "classical" markers did not appear in the US literature until the early 1970s, when a series of articles authored by M.G. Weiss were published that documented seven soybean linkage groups comprising a total of 18 of the (then) known 80 soybean classical markers (cf. Bernard and Weiss, 1973). By 1984, the number of CLGs had nearly doubled to 13 and involved a total of 31 of the (then) known 164 markers, of which 133 were classical markers and 31 were allozyme markers (Palmer and Kilen, 1987). The allozyme markers were treated (at the time) as "pseudo molecular" markers in the sense that these were enzymatic proteins rather than traits per se. In the most recent soybean monograph documenting advances in multiple soybean research areas (Boerma and Specht, 2004), Palmer et al. (2004) listed 21 CLGs, which included a total of 70 markers (classical and allozyme) of the 264 total existent marker loci tabulated in their chapter. It is of interest to summarize here the nature of these 264 loci by classical category: pest reaction/response (43), herbicide response (6), nodulation (8), root-related traits (8), above-ground morphology/development (53), fertility/sterility (20), physiological traits (11), leaf/seed chlorophyll (28), pigmentation (16), allozymes or proteins (58), and fatty acids (13). At present, there are 478 known classical, allozyme, and other genetic markers, and a list of these can be found in SoyBase (http://soybase.org; verified April 7, 2012), by clicking on the Maps tab, then on the Genes tab.

The article published by Botstein et al. (1980), who described how to use restriction fragment length polymorphism (RFLP) markers to create a human genetic map, stimulated the development of such maps in soybean. The first soybean map based on RFLP markers was published 10 years later (Keim et al., 1990). More comprehensive public and proprietary genetic maps with

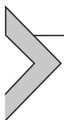
molecular linkage groups (MLGs) were subsequently published (Rafalski and Tingey, 1993; Shoemaker and Olson, 1993). These first RFLP maps were constructed by using *G. max* × *G. soja* populations because the number of RFLP markers for soybean was limited at that time and an interspecific mating offered more opportunity for RFLP polymorphism (Keim et al., 1989, 1990, 1992). Many of the classical genes in soybean were backcross-introgressed into one or more of three soybean cultivars (Clark, Harosoy, Williams), and the resulting near-isogenic lines have been preserved in the soybean germplasm collection. Muehlbauer et al. (1988) pointed out that the Recurrent Parent/NIL/Donor Parent triplet sets could be used for integrating the classical and molecular marker maps. Because of the multiplicity of markers in molecular maps, classical marker maps could be merged into the former (Shoemaker and Specht, 1995). However, RFLP markers were cumbersome to create and use, and soon after the polymerase chain reaction (PCR) was reported, soybean geneticists started using PCR-based markers such as simple sequence repeats (SSRs), random amplification of polymorphic DNA (RAPDs), AP-PCRs, DNA amplification fingerprinting (DAFs), and amplified fragment length polymorphisms (AFLPs). Although all these markers were used at some time for the creation of some maps, the SSR marker turned out to be the marker of choice for the construction of dense linkage maps, mainly because these markers were single locus, potentially multiallelic on a per locus basis, and randomly distributed in the genome. Indeed, the use of SSR markers allowed, for the first time, the creation of a genetic map of just 20 MLGs—a number consistent with the number of soybean chromosomes (Cregan et al., 1999). In the decade that followed, SSRs were used to construct many genetic maps. Shoemaker et al. (2004) provided a table summarizing the (then) available mapping populations of all the foregoing marker types, and Cregan (2008) later provided an updated list. The reader can find a current list of all mapping populations reported to date for all of the foregoing marker types in SoyBase (http://soybase.org; verified April 7, 2012), by clicking on the Maps tab, and then on the Experiment-specific Maps tab.

Recently, Song et al. (2010) pointed out that there are hundreds of thousands of potential SSR loci in the Williams 82 genome sequence, and they identified the genome position and primer sequences needed to amplify 33,065 of these SSRs. This is a very useful resource, particularly during the early stages of a gene-cloning project when markers are needed to identify recombination events in a gene-rich region encompassing many candidate genes. Although SSRs have proven to be a useful marker for many purposes, multiplexed SSR marker screening of a large number of existent genotypes in a germplasm collection or segregants in a mapping population is not

effectively possible—except on a limited scale using time-of-flight mass spectrophotometry for SSR allele sizing (Shoemaker et al., 2004). Large-scale SSR genotyping with slab-gel or capillary electrophoresis is a cumbersome and time-consuming process.

With the advent of single-nucleotide polymorphism (SNP) markers (Collins et al., 1998), soybean geneticists now have an alternative to SSRs for large-scale genotype screening. SNP markers are potentially much more abundant than are SSRs markers in the genome (Zhu et al., 2003). Choi et al. (2007) published the first comprehensive soybean genetic map that included 1361 SNP markers in addition to 634 RFLP and 983 SSR markers. The most recent soybean molecular map, known as Version 4.0, was facilitated by the creation of a 1536-SNP marker chip and its use as an SNP marker linkage panel in multiple mapping populations that had been previously genotyped for other types of markers (Hyten et al., 2010). At present, this 1536-SNP chip is the marker resource of choice for soybean breeders and geneticists for genotyping mapping populations developed to detect quantitative trait loci (QTLs). In general, 300–600 of the 1536-SNP markers on this chip are parentally bimorphic in any given population, with the actual number depending on the diversity of the parents used in the mating that generated the mapping population. Larger multiplexed SNP marker systems are being developed and are addressed in Section 3.2.

The ultimate goal of genetics is to connect genotype with phenotype. The detection of QTLs for various traits of economic and scientific value was made possible by the advent of markers and marker linkage maps. Many soybean QTLs have been discovered since the first publications on QTL discovery appeared in the early 1990s (Diers et al., 1992; Keim et al., 1990). All the QTLs discovered to date are listed in SoyBase (http://soybase.org; verified April 7, 2012), and can be accessed by clicking on the Maps tab, then on the QTL tab.



3. THE GENOMIC ERA

An early draft of the soybean genome sequence was first made public in 2008, whereas the finalized sequence and accompanying journal article with comprehensive analyses was published in 2010 (Schmutz et al., 2010). This was a tremendous step forward for the research community, as the genome sequence resource enabled several immediate advancements. For example, the genome sequence integrated the soybean genetic and physical maps, giving an insight into the size of the linkage blocks associated with

any given marker QTL. Further, the genome sequence annotation defined the positions of protein-encoding genes, making possible the identification of candidate genes in QTL cloning projects. Moreover, the reference genome facilitated the development of more extensive and efficient marker platforms and new markers that can be used for user-specific applications, such as fine mapping within a given genetic interval. All these applications are continuously being refined.

Beyond its utility as a reference, the genome sequence is a rich reservoir of molecular genetic information. This section delves into the primary discoveries that accompanied the sequencing and resequencing of soybean and its wild relative *G. soja*.

3.1. What's in the Soybean Genome?

The reference soybean genome sequence serves as a catalog of soybean gene content and other interesting features of the genome (Schmutz et al., 2010). Although several other legume genomes have been sequenced to date, to varying degrees of completion (Sato et al., 2008; Varshney et al., 2012; Young et al., 2011), the soybean genome is the most complete, comprising 950 million base pairs of an estimated 1.1 billion base pair genome. There are approximately 46,000 high-confidence protein-coding gene models predicted in the soybean genome (an additional ~20,000 loci were predicted with lower confidence), 75% of which are represented by at least two or more copies. Gene duplication is a signature feature of the soybean genome that pervades and sometimes complicates genome structural and functional analysis. Many of the duplicated soybean genes reside within ancient homoeologous blocks, and are syntenic remnants of ancient whole-genome duplications estimated to have occurred once approximately 59 million years ago (mya), and then again approximately 13 mya. The other sequenced legume genomes share the 59-mya duplication event, as this event occurred at the root of the large Papilionoideae subfamily (Cannon et al., 2006, 2010). However, the duplication 13 mya is unique to soybean among the sequenced legumes. Interestingly, the other legume genomes seem to have a similar total number of genes as soybean, despite lacking the more recent whole-genome duplication event (Young and Bharti, 2012).

The soybean genome exhibits a surprisingly organized landscape. The 20 chromosomes range from approximately 38 to 60 million base pairs each. The inner space of each chromosome is highly heterochromatic, including a vast collection of repetitive DNA sequences such as transposons and retrotransposons (Schmutz et al., 2010). The transposon content of the soybean

genome has been compiled into the SoyTEdb database (http://soybase.org/soytedb/; Du et al., 2010). The sequences that are located at the centromere position of the chromosomes (the primary constriction) consist of two related tandem satellite repeats, including a 92- and a 91-bp monomeric form (Gill et al., 2009). The 92-bp repeat localizes to 14 of the 20 chromosome pairs, whereas the 91-bp repeat localizes to eight chromosome pairs (the two repeats colocalize to two pairs of chromosomes). The existence of two primarily nonoverlapping centromere satellite repeats has been offered as evidence that the genome duplication event 13 mya resulted from interspecific hybridization of two different *Glycine* species, thereby making modern soybean an ancient allopolyploid species (Gill et al., 2009). There is experimental evidence that these satellite repeats are in fact localized to the functional centromeres per se, as their chromatin is associated with the putative centromere-specific histone protein GmCenH3 (Tek et al., 2010). Further, two other classes of repetitive DNA elements were also found to associate with the GmCenH3 histone.

The outer euchromatin chromosomal arms, which comprise approximately 43% of the sequenced space, contain the majority of the soybean genes. These gene-rich regions are less densely populated by repetitive sequences and experience much higher rates of genetic recombination (Schmutz et al., 2010). As mentioned above, the majority of the genes are present in more than one copy, oftentimes arranged as blocks of duplicated genes across different chromosomes. The block sizes are highly variable in terms of duplicate conservation and size, ranging from 6 to 736 gene pairs. Within the blocks, duplicated gene pairs are interspersed with noncollinear genes that do not have a matching paralog. Some of the duplicated blocks have been studied in detail. For example, Innes et al. (2008) performed a detailed analysis of a duplicated block that encompassed the *Rpg1-b* disease resistance gene. They reported a high conservation of gene retention between blocks, despite a 3-fold expansion in sequence space experienced by one of the blocks due to retrotransposon accumulation. Similarly, Lin et al. (2010) reported on the divergence between a 1-megabase duplicated block located on soybean chromosomes 8 and 15. A high gene retention rate between blocks was also noted in this study, despite there being different retrotransposon compositions and a large inversion event in the chromosome 15 block. In addition to the inversion, chromosome 15 also exhibited a higher retrotransposon density, and the genes in this block generally exhibited lower transcription rates than did their duplicate pairs on chromosome 8. Further, Severin et al. (2011) recently examined a duplicated

block that is conserved at 12 different locations in the soybean genome. The authors concluded that the 12 blocks were created by three rounds of whole-genome duplication, including the events from approximately 13 mya, approximately 59 mya, and also a more ancient event from approximately130 to 240 mya (Severin et al., 2011).

Not all paralogous gene copies in the soybean genome resulted from whole-genome duplications. The soybean genome is also populated by numerous dispersed and tandemly arranged gene duplications. Some notable gene families that have been greatly expanded by tandem duplication include defense gene clusters and genes encoding F-box and auxin-responsive protein domains (Schmutz et al., 2010). Similar findings have been reported in other plant genomes.

Beyond the descriptive aspects and the patterns observed, the soybean genome sequence and its associated gene annotations are a ready-to-use reference for identifying genes of great interest and/or value. The genome sequence has already been used to accelerate the cloning of several genes that influence important traits (e.g. Cook et al., 2012; Liu et al., 2012; Meyer et al., 2009; Saghai Maroof et al., 2009; Skoneczka et al., 2009). Tian et al. (2010) provided one particularly nice example of using the soybean genome sequence to accelerate the discovery of a gene responsible for differentiating between determinate and indeterminate growth habit. In this study, the authors leveraged information from the model plant species *Arabidopsis thaliana*, which had a previously characterized mutation known as *tfl1* that exhibited determinate habit (*A. thaliana* is normally indeterminate). The authors investigated the soybean genome sequence and found four soybean gene models that are homologous to the *A. thaliana TFL1* gene. The authors used information from a published soybean genetic mapping study (Liu et al., 2007) to select the gene model that co-localized to the determinate QTL region. Further functional and association analysis supported that this gene is in fact the determinacy gene Dt1 (Bernard, 1972; Liu et al., 2010a; Tian et al., 2010). This is an excellent example of genome-enabled translational genomics, whereby information from a model species and the soybean genome sequence were used to characterize gene function in soybean.

3.2. Genome Resequencing

The soybean reference genome sequence has enabled new research opportunities in the areas of germplasm diversity and comparative genomics. It has particularly enabled genome-resequencing studies, whereby deep sequencing data from a series of soybean accessions or wild relatives can be aligned

to the reference to identify sequence nucleotide variants (e.g. SNPs) and/or larger structural variants (e.g. deletions, duplications, insertions, inversions, or chromosomal translocations greater than 1000 nucleotides in length). Resequencing studies are critical in crops species because they reveal new insights into the genetic limitations of the domesticated germplasm and the potential for using wild germplasm.

The wild soybean *G. soja* genome was sequenced to approximately 43-fold genome coverage and assembled based on the *G. max* reference (Kim et al., 2010). Kim et al. (2010) sequenced one *G. soja* individual, accession number IT182932, and cataloged the range of sequence variation observed with the soybean reference genome. They identified over 10,000 genes exhibiting SNPs and/or small insertion/deletion polymorphisms that might cause differences in protein function between the two species. Further, the authors documented approximately 1,000 genes that were putatively associated with genomic structural variants. The majority of such instances involved a gene that was present in *G. max* and not present in *G. soja*, implying that the gene had either been deleted in *G. soja* or evolved de novo in the *G. soja*–*G. max* lineage sometime following the split with *G. soja* accession IT182932. A smaller portion of these genes was identified with chromosomal inversions between the two species.

As mentioned above, soybean is believed to have been domesticated from *G. soja* 3500 years ago (Hymowitz, 2004). Kim et al. (2010) estimated the divergence time between the soybean reference genome and the *G. soja* accession IT182932 as approximately 270,000 years ago. Although perhaps counterintuitive, these vastly different time scales do not necessarily contradict one another. The estimated time of divergence between a domesticate and just one wild progenitor will generally predate the time of domestication, as it reflects the time to common ancestry shared between the two lineages rather than the time of domestication (Morrell et al., 2012). Thus, these two divergence time estimates reflect different aspects of soybean history.

Lam et al. (2010) performed lower-coverage resequencing on a deeper panel of accessions, consisting of 14 *G. max* and 17 *G. soja* accessions sequenced to approximately fivefold genome coverage. The authors focused their analysis on describing population-level phenomena, particularly the genomic regions that exhibited conserved polymorphisms between the domesticated and wild accessions. The authors reported a higher allelic diversity within *G. soja* than within *G. max*, and provided a subset of SNP markers that, based on linkage disequilibrium (LD) block distribution, they

predict will be useful for QTL mapping and association studies. In terms of genomic structural variation, Kim et al. (2010) and Lam et al. (2010) both estimated that there are approximately 1,000 genes located within structural variants between *G. max* and *G. soja* (Stupar, 2010). Further, Kim et al. (2010) and Lam et al. (2010) both identified subsets of genes present in *G. soja* that were not found in the *G. max* reference sequence. These findings further substantiate the notion that wild soybean is a resource for both novel alleles and novel genes that are not currently available in the domesticated germplasm.

The studies summarized above represent the tip of the iceberg for soybean resequencing. Several resequencing projects are currently under way, including a study focusing on a 1008 accessions (H.T. Nguyen, personal communication) and a study focusing on breeder selection in the twentieth century by resequencing the North American ancestors and a set of milestone public cultivar releases (R.C. Shoemaker and J.E. Specht, personal communication).



4. THE POSTGENOMIC ERA

Deciphering the genetic blueprint that encodes a soybean plant is a fascinating discovery in itself. Today, a major area of interest for the postgenomic soybean community is using the reference genome as a basis for assessing genetic diversity in the germplasm, understanding how genes function and applying these insights to soybean improvement.

4.1. Germplasm Haplotyping and Association Mapping

Following the completion of the soybean genome, genetic tools and resources have become the new limitation to identifying the genetic polymorphisms that drive trait variation. Many of these genetic tools are currently being developed. This section addresses recent advancements in genotyping technologies and genetic population development that are geared toward enhancing the resolution of genetic mapping in soybean.

As was addressed in Section 2.3, genetic mapping in soybean has historically relied on a progressive series of gel electrophoresis-based platforms. Soybean SNP-based genotyping platforms have rapidly advanced in the past few years, accelerating genetic mapping studies and raising the prospects for association mapping. In Section 2.3, we noted that the SNP-based platform preferred by soybean breeders and geneticists for QTL detection is the soybean 1536-SNP panel (Hyten et al., 2010). The 1536 platform has

been used for numerous studies including genetic mapping of rust resistance (Hyten et al., 2009), soybean cyst nematode (Kim et al., 2011), innate immunity responses (Valdes-Lopez et al., 2011), and several other traits. Genotyping by sequencing is a relatively new tool that may soon become available to breeders (due to rapidly falling sequencing costs) for use in multimarker genomic selection projects (Elshire et al., 2011). Sequence-based genotyping approaches have worked well for soybean studies with very limited samples sizes (Severin et al., 2010a; Varala et al., 2011), but have not been published to date for soybean breeding and/or QTL projects.

Multiplexed marker systems have now been enhanced to much greater numbers, including a 50K SNP chip. To date, this platform has only been used in one publication, a study that investigated genetic diversity within cultivar Williams 82 (Haun et al., 2011). However, the 50K SNP chip was recently used to genotype the entire *G. max* and *G. soja* collection held by the USDA. Although the data are not net summarized for public use (P.B. Cregan, personal communication), this genotyping effort of nearly 20,000 accessions will vastly dwarf all the previous assessments of soybean genetic diversity. This dataset will enable the development of high-resolution haplotypes for each accession in the collection, which can be used to classify the genetic relationship among accessions and refine assessments of LD. Previous assessments indicate that soybean has a high level of LD (Hyten et al., 2007; Lam et al., 2010), as may be expected from a selfing species that experienced a strong domestication bottleneck (Hyten et al., 2006). The high level of LD in soybean will be advantageous for genomewide association studies, but will limit the utility of association mapping for gene-cloning efforts or other applications requiring high mapping resolution.

Recently, a Nested Association Mapping (NAM) population has been developed for soybean that promises to increase mapping resolution and contribute a great deal of new knowledge regarding the genetic architecture of quantitative traits. The NAM approach involves mating a diverse subset of parental lines to a common "hub" parent and then self-pollinating each lineage into recombinant inbred line (RIL) populations. The NAM approach, as constructed in maize, was developed to combine the advantages of traditional linkage analysis and association mapping. The NAM design uses both historic and recent recombination events, allowing for joint-linkage-association mapping within the population (McMullen et al., 2009) and adding statistical power and resolution for mapping polymorphisms that drive phenotypic variation. Recently, the maize community has used the NAM approach for the high-resolution mapping of both small

and large-effect QTL for highly polygenic traits, such as flowering time (Buckler et al., 2009), leaf architecture (Tian et al., 2011), and Southern leaf blight (Kump et al., 2011).

The 41 parental genotypes selected to develop the soybean NAM population represent a similarly diverse range of germplasm (Table 4.1). Forty lines were mated with a hub parent (IA 3023) and subsequently self-pollinated to generate 140 RILs per cross. The parental lines are being resequenced, and the RILs are being genotyped using a 6K SNP platform. The SNPs identified by resequencing can be imputed onto the RIL individuals, providing high-resolution genotyping across the population. The soybean NAM population is currently being phenotyped for agronomic traits. In 2011, Brian Diers and Jim Specht conducted single-replicate NE and IL agronomic trials (yield, maturity, plant height and lodging, 100-seed weight, seed protein, and oil) on 70 of the RILs of the NAM populations. In 2012 and 2013, all 140 RILs of each of the 40 NAM matings will be evaluated in single-rep trials in NE, IA, IL, IN; with partial replicates grown elsewhere in MO, OH, MI. The 41 parents are available to researchers interested in characterizing the lines for an agronomic or other trait of interest. If one or more biparental contrasts are found, then those researchers can request RIL seed, or make direct observations in one of the agronomic trials conducted in the foregoing states in 2012 and 2013.

4.2. Genomic Structural Variation within Soybean

Molecular diversity in plant genomes is frequently considered at the scale of single-nucleotide changes or transposable element activity. However, recent studies in human and other animal species have focused on structural genomic alterations, such as large deletions, duplications, inversions and translocations, as potential drivers of phenotypic variation (Stankiewicz and Lupski, 2010). Recently, genomewide structural variation analyses has been performed in crop species, such as maize (Belo et al., 2010; Springer et al., 2009; Swanson-Wagner et al., 2010), rice (Yu et al., 2011), and sorghum (Zheng et al., 2011). Further, some studies have reported that structural variation for a specific gene is associated with crop trait variation, such as winter hardiness in barley (Knox et al., 2010) and dwarfism in wheat (Pearce et al., 2011). One of the new frontiers for plant genomics will be cataloging and interpreting structural variation data, and learning how to use this information for breeding purposes.

Structural variation comparisons between soybean and *G. soja* are addressed in Section 3.2. However, structural variation among soybean

Table 4.1 Accessions Used to Create the Soybean NAM Population

NAM Pop.	Parentage	Maturity*	NAM Pop.	Parentage	Maturity*
2	TN05-3027 × IA 3023	V	27	IA 3023 × LG05-4292	IV
3	IA 3023 × 4J105-3-4	III	28	IA 3023 × LG05-4317	IV
4	IA 3023 × 5M20-2-5-2	III	29	IA 3023 × LG05-4464	III
5	IA 3023 × CL0J095-4-6	III	30	IA 3023 × LG05-4832	III
6	IA 3023 × CL0J173-6-8	III	31	IA 3023 × LG90-2550	III
8	HS6-3976 × IA 3023	III	32	IA 3023 × LG92-1255	II
9	IA 3023 × Prohio	III	33	IA 3023 × LG94-1128	II
10	IA 3023 × LD00-3309	IV	34	IA 3023 × LG94-1906	II
11	IA 3023 × LD01-5907	IV	36	IA 3023 × LG97-7012	III
12	IA 3023 × LD02-4485	III	37	IA 3023 × LG98-1605	III
13	IA 3023 × LD02-9050	IV	38	IA 3023 × LG00-3372	III
14	IA 3023 × Magellan	IV	39	IA 3023 × LG04-6000	IV
15	IA 3023 × Maverick	IV	40	IA 3023 × PI 398.881	III
17	IA 3023 × S06-13640	IV	41	IA 3023 × PI 427.136	III
18	IA 3023 × NE3001	III	42	IA 3023 × PI 437.169B	II
22	IA 3023 × Skylla	III	46	IA 3023 × PI 507.681B	II
23	IA 3023 × U03-100612	II	48	IA 3023 × PI 518.751	II
24	IA 3023 × LG03-2979	III	50	IA 3023 × PI 561.370	III
25	IA 3023 × LG03-3191	IV	54	IA 3023 × PI 404.188A	II
26	IA 3023 × LG04-4717	III	64	IA 3023 × PI 574.486	II

*Indicates the maturity group of the nonhub parent. The hub parent IA 3023 is in maturity group III.

accessions in the *G. max* germplasm collections (i.e. intraspecific comparisons) may be the most meaningful from an applied research standpoint, as this represents the gene pool most frequently used by breeding programs. Genomewide assessments of structural variation among soybean accessions have been recently performed by using a comparative genomic hybridization (CGH) microarray platform and resequencing of exon-enriched sequences from the genome (Haun et al., 2011; McHale et al., 2012). These approaches are particularly effective for identifying genes and other sequences that are present in the reference genotype Williams 82 but either missing or highly polymorphic in other accessions. These studies looked at a small subset of accessions to assess the general trends of structural variation in soybean. One major finding was that defense gene clusters, namely, genes containing a Nucleotide Binding domain or predicted to encode a receptor-like protein, were highly enriched for structural variation and/or high rates of nucleotide polymorphism. These gene classes are typically associated with plant pathogen perception and signaling of defense responses, and are frequently referred to as resistance gene analogs (RGAs). A recent study noted that there is a large degree of variation among soybean accessions for RGA abundance (Zhang et al., 2010). Further, sequence-based comparisons have revealed several predicted genes within defense gene clusters that exhibit copy number or present–absent variation among soybean accessions (Cook et al., 2012; M.A. Graham; B.W. Diers, personal communications).

Thus, although the soybean domestication bottleneck would suggest a limited diversity in *G. max* for most genes and gene families vis-a-vis the potentially greater diversity in *G. soja*, defense-related genes may be an exception. There may be several hundred or even thousands of yet undiscovered RGAs in just the domesticated soybean germplasm. It is also noteworthy that genes involved in immunity and environmental responses have also been associated with structural variation hotspots among human and other mammalian genomes (Gokcumen et al., 2011; Liu et al., 2010b), suggesting that this may be a generalizable phenomenon throughout eukaryote genomes.

4.3. Gene Function

Up to this point, this chapter has almost exclusively addressed topics relevant to natural variation in the soybean germplasm. However, an in-depth understanding of gene function is the primary goal for many plant genomicists. The soybean genome revealed high-confidence models for approximately 46,000 genes, most of which have an uncharacterized function. In many cases, a soybean gene can be initially characterized based on known

functions of similar genes that have been studied in model species. However, this is of limited utility, particularly in the case of a highly duplicated genome such as soybean, where gene function may be redundant among copies, or become subfunctionalized or neofunctionalized among copies (Force et al., 1999; Prince and Pickett, 2002), which, of course, cannot be inferred from sequence data alone. Perhaps, the greatest current limitation facing soybean functional genomics is the development of comprehensive genetic and genomic tools.

The most straightforward approach toward understanding gene function is to develop mutations in the coding region of a specific gene of interest. In many cases, the mutation will lead to a loss of function for the gene, thus allowing for an assessment the plant phenotype with and without the specific gene. Model genetic systems such as maize and *A. thaliana* have extensive mutant resources to advance functional genomic investigations and applications in these species (Alonso et al., 2003; Strable and Scanlon, 2009).

The soybean community has also developed mutant resources and is continuing to do so. The two major classes of mutations that have been induced to date include chemical and irradiation-based mutagenesis. In the case of chemical mutagenesis, small DNA base changes are induced that can alter or eliminate gene function and plants can be phenotyped to detect specific alterations of interests (Carroll et al., 1985; Hoffman et al., 1999). One of the main advantages of this approach is that the DNA from the mutant population can be pooled and screened to identify new alleles for specific genes. This is particularly useful if a researcher is interested in a trait that is difficult or expensive to phenotype. Essentially, the researcher can selectively screen for DNA changes in nearly any one of the 46,000 soybean genes using a PCR-based Targeting Induced Local Lesions in Genomes (TILLING) method. This was successfully accomplished in soybean to identify 116 induced mutations detected in populations derived from either ethyl methanesulfonate (EMS) or *N*-nitroso-*N*-methylurea chemigans (Cooper et al., 2008). TILLING of an EMS population of Williams 82 identified new alleles for the *RS2* and *FAD2-1A* genes, resulting in altered seed meal and oil composition traits (Dierking and Bilyeu, 2009). Although the screening process is manual and laborious, the promising developments arising from pooled resequencing methodologies (Tsai et al., 2011) may eventually alleviate this limitation.

Irradiation-based mutagenesis is primarily used for developing mutated lines with DNA segments that have been deleted. Interesting soybean mutant lines induced by X-ray and fast neutron irradiation have been reported,

including the increased oleate line M23 (Rahman et al., 1994) and a supernodulating line (Men et al., 2002). Genotyping of irradiation mutants using CGH microarrays has led to the physical mapping of specific chromosomal deletions (and duplications) within irradiated mutant lines (Bolon et al., 2011). Subsequent PCR analysis of candidate deletions within families segregating for the mutant phenotype has revealed deletion–trait associations. For example, the increased oleate line M23 mutant phenotype was found to cosegregate with a 164-kb deletion on chromosome 10, and a dominant short petiole mutant phenotype identified in a fast neutron population was found to cosegregate with an 838-kb deletion on chromosome 17 (Bolon et al., 2011). In both cases, the exact deletion breakpoints were mapped by sequencing the PCR amplicons from the respective mutated lines.

One of the fortunate byproducts of the CGH/PCR analysis pipeline for irradiated mutants is that a PCR-based marker is concordantly developed that can predict the mutant phenotype. For instance, in the case of M23, there is no obvious visual marker for the increased oleate trait and heterozygous individuals cannot be reliably selected on the basis of oleate content (Alt et al., 2005). However, the PCR marker could be used by a breeder to rapidly introgress this locus into an elite genetic background. The main limitation of the CGH/PCR approach is throughput; only a very finite number of lines can be genotyped with this platform, rendering irradiation mutagenesis solely a forward genetics approach. However, as stated above with regard to chemical mutagenesis, there is the possibility that genome-resequencing approaches may enable the screening of larger, pooled populations to identify potentially useful lesions, thereby reducing the burden of phenotypic screening for some genes and/or traits. Further, a resequencing-based approach using a large number of individuals will increase the likelihood of identifying small deletions of interest, as it seems that forward genetic screens have a tendency to identify relatively large deletions, which may be less desirable from a breeding perspective.

Several successful mutant populations in maize have been developed from the mobilization of transposable elements, which can insert into a gene of interest thereby eliminating function. Natural rates of DNA transposition in soybean are insufficient for generating transposon tagged mutants using conventional germplasm. However, recent reports indicate that transposon-based mutagenesis can be established by genetically transforming the components for transposable element activity into soybean. The maize *Dissociation* (*Ds*) transposon system (Vollbrecht et al., 2010) has been transformed into soybean and shown to mobilize and disrupt gene function,

including one example in which a strictosidine synthase gene was disrupted (Mathieu et al., 2009). Further, the Ds system is also being developed as an activator tagging system, wherein the transposon is linked to transcriptional enhancers that are capable of upregulating nearby genes (resulting in gain of function rather than loss of function mutations). The rice *mPing* system (Jiang et al., 2003) is also being developed as a transposon tagging system for gene disruption. Early work on mPing indicate that this is a promising system, as heritable *mPing* transpositions in soybean show a preference for inserting nearby genes (Hancock et al., 2011). Further, there are ongoing efforts to develop a soybean mutant population tagged with the tobacco element *Tnt1*, as has been developed in *Medicago truncatula* (d'Erfurth et al., 2003).

Posttranscriptional gene silencing (PTGS) methods can be used to suppress gene products in specific environmental conditions or developmental stages. One method uses transgenic RNA interference (RNAi) constructs, under the control of a specific promoter, to trigger gene silencing in certain conditions or stages of development. In soybean, this approach has been used to specifically silence *FAD2* gene family members, resulting in increased oleic-acid levels in the seed (Wagner et al., 2011). Further, virus-induced gene silencing (VIGS) offers a nontransgenic approach for gene silencing during a specific developmental stage. In soybean, a bean pod mottle virus-based vector has been modified to silence the transcription of a specified gene on soybean infection. This approach has been used to characterize the function of several genes involved in fatty acid biosynthesis and plant defense responses (Kachroo et al., 2008; Liu et al., 2011; Meyer et al., 2009). One advantage of the PTGS methods is that they are capable of simultaneously targeting multiple members of multicopy gene families, which may be critical for observing a phenotype that may be masked in mutant lines due to functional redundancies among the copies. Alternatively, this may also be viewed as a disadvantage, if the research question of interest requires the silencing of only one specific gene copy. Therefore, the reference genome sequence is a critical tool for construct design, as areas of high or low conservation among gene copies may be targeted depending on the objectives of a given experiment.

A suite of genomic tools collectively referred to as "genome engineering" are now emerging that may eventually alter the landscape of crop functional genomics. These technologies are based around the process of engineering DNA double-stranded breaks at specific locations to induce repair events with altered DNA sequences. This technology has been pioneered in human cell lines and model plant and animal species (Carroll,

2011; Lee et al., 2012), but has also been used to generate site-specific changes in tobacco, maize and petunia (Marton et al., 2010; Shukla et al., 2009; Townsend et al., 2009; Tzfira et al., 2012). These genome-engineering technologies are capable of generating gene-specific mutations, deletions, inversions, translocations and, most intriguingly, gene editing. Indeed, gene-specific mutations have already been developed in soybean using this approach (Curtin et al., 2011; Sander et al., 2011). Currently, the development of stable mutated lines is relatively slow, as it requires genetic transformation and extensive segregation analysis of transformed materials. Still, the potential to perform targeted gene editing, whereby gene sequences can be altered with specific (rather than random) modifications, makes this one of the most exciting areas of genetics research.

It is important to note that each and every one of the methods described above is enhanced by using the soybean reference genome sequence. For example, the genome sequence enabled the construction of a CGH platform that can be used to genotype deletions and duplications in irradiated mutant lines. Further, as mentioned above, the genome sequence can be used to design RNAi constructs or VIGS vectors that target specific genes or multigene families. The same can be said for the full suite of soybean genome-engineering technologies.

4.4. Beyond the Genome

Although the purpose of this chapter is to address the state of the soybean genomic research community, it is important to also mention that this research exists within a synergistic framework involving the other branches of high-throughput "-omics" research. For example, recent developments in soybean RNA-Seq and transcriptomic analyses led to the development of the soybean gene expression altas (Libault et al., 2010; Severin et al., 2010b; Woody et al., 2011), which gave a new insight into the expression profiles of the vast majority of the approximately 46,000 genes across different stages of soybean development. Similar advances are ongoing within the epigenomic (Kulcheski et al., 2011; Li et al., 2011), proteomic (Brechenmacher et al., 2009), metabolomic (Brechenmacher et al., 2010), and methylomic (S.A. Jackson, personal communication) realms. Perhaps most importantly, the soybean community awaits breakthroughs in plant phenomics, whereby high-throughput phenotyping can be realized. Advances within all of these fields are critical for advancing the "systems biology" of soybean, where an understanding of the complex interactions among disciplines can be used to model the soybean plant as a whole.

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CHAPTER FIVE

Responses of Crop Plants to Ammonium and Nitrate N

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Contents

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Abstract

Nitrogen (N) is the most important, essential nutrient for all living organisms on earth; it is present in a number of complex organic molecules and plays extremely important roles in their activities. Ammonium N (NH_4^+–N) and nitrate N (NO_3^-–N) are the main forms taken up by plants in addition to some organic N compounds.

More than 90% soil N is in organic form. The intermediate products of complicated organic N substances can be absorbed by plants. Organic N nutrition affects plant product quality and plant metabolism. Organic N passes through the cell wall and arrives at the plasma membrane through the apoplast and cytoplast systems and, in addition to endocytosis, may get transported across the plasma membrane by an active (sugar/proton cotransport) or passive process. After uptake by plants, simple organic N compounds such as amino acids can be rapidly assimilated and transformed into other amino acids by transamination and deamination.

The uptake of NH_4^+–N and NO_3^-–N can be described by the Michaelis–Menten equation, and two parameters, the maximum absorption velocity (*V*max) and affin-

ity constant or Michaelis constant (*K*m), have been used to measure the ability and efficiency of roots absorbing the two ions of crop plants. The uptake amounts of both NH_4^+–N and NO_3^-–N at the seedling stage are well in agreement with their absorption kinetic parameters, particularly at low concentrations, but are not fully in agreement with the entire growing periods of crops.

In addition to root interception, NH_4^+ and NO_3^- can move from bulk soil to the root surface by mass flow and diffusion. Diffusion is more important to NH_4^+–N than NO_3^-–N, while NO_3^-–N movement mainly depends on mass flow.

Roots are the major organs for the uptake of NH_4^+ and NO_3^- ions. On arriving at the root surface, the two N forms can passively enter the root epidermis cell wall through the symplast and apoplast and then radically and vertically move across the cortex where the two ions in the apoplast enter the cortex symplast for passing through the endodermal Casparian trip to the endodermis. From the endodermis, the two ions go to the stele and empty into the xylem, or flow to the apoplast or get stored in vacuoles in addition to reduction or direct assimilation. The movement occurs from cell to cell. On emptying into the xylem, NH_4^+ and NO_3^- are transported to the shoots via the transpiration stream.

NO_3^-–N is the dominant form of the mineral N with high concentrations in soil solutions, and is usually taken up in great amounts by crops and is readily mobile in the xylem. Transport of NO_3^- across the plasma membrane along the electrochemical gradient is thought to be by H^+/NO_3^- cotransport, or by transport proteins or carriers or by specific ion channels.

NH_4^+ is in equilibrium with NH_3, and in most soils, the pH is considerably low and NH_3 concentrations are usually very low. NH_4^+ uptake through the plasma membrane has been assumed to occur in three ways: either active or passive, or both. Passive uptake may occur at the initial stage of uptake, while at the second stage, active uptake may be predominating. For passive uptake, NH_4^+ ions passing through the membrane are thought to be present in either NH_4^+ or NH_3 form, and in this way may be related to the facilitated diffusion through channels. The NH_4^+ ion resembles the K^+ ion in terms of the ionic radius and size of the hydration shell, and therefore, it may be able to permeate the plasma membrane through K^+ channels. The active uptake includes the H^+/NH_4^+ cotransport, and specific transporters.

Nitrate N cannot be directly used by plants until it is reduced to ammonia. The reduction is catalyzed by enzymes in two steps: the first step takes place in the cytoplasm by nitrate reductase (NR) transforming NO_3^- into nitrous acid (HNO_2), and the second occurs in chloroplasts (shoots) or proplastids (roots) by nitrite reductase (NiR) converting HNO_2 to NH_3. The NO_3^- reduction to NO_2^- is the rate-limiting step for NO_3^- assimilation. Both roots and shoots are capable of reducing NO_3^-. The uptake and reduction of NO_3^- and the reductive ratios in roots and shoots depend on plant species, carbohydrates in plants, and nitrate reductase activity (NRA) as well as environmental conditions such as NO_3^- concentration, medium pH, complementary ions, light, and ambient CO_2 concentration. Due mainly to NO_3^- uptake exceeding its assimilation by plants, a large amount of NO_3^- is accumulated in vacuoles of roots and shoots at vegetative stages. With the exception of vegetables, the storage of NO_3^- has no harmful effects on plant product quality and benefits the supply of N nutrient for later growing stages of plants.

Ammonia is a central intermediate in plant N metabolism. NH_3 is assimilated by plants by the mediation of glutamine synthetase–glutamine (z-) oxoglutarate aminotransferase enzyme systems in two steps: the first step requires adenosine triphosphate to add NH_3 to glutamate to form glutamine (Gln), and the second step transfers the NH_3 from Gln to α-ketoglutarate to form two glutamates. Once NH_3 has been incorporated into glutamate, it can be transferred to other carbon skeletons by various transminases to form additional amino acids. The glutamate and Gln formed can rapidly be used for the synthesis of low-molecular-weight organic N compounds (LMWONCs) (such as other amides, amino acids, ureides, amines, and peptides) that will be further synthesized into high-molecular-weight organic N compounds (HMWONCs) such as proteins and nucleic acids.

NH_4^+–N and NO_3^-–N may exhibit different effects on plant nutrition, growth, and crop production of different plant species. The preferences are the demand nature of plant species together with environmental conditions. The preferences have been evaluated mainly by plant biomass, yield production, or N uptake amount by the application of NH_4^+–N or NO_3^-–N alone. Based on current results, crop plants may be classified into four types: preference to NH_4^+–N; preferences to NO_3^-–N; equal effect of NH_4^+–N and NO_3^-–N; combinative use of the two N sources being superior to either NH_4^+ or NO_3^- alone.

Based on the fact that when NH_4^+–N and NO_3^-–N coexist in media, crops have a higher dry matter production, N uptake, and N utilization efficiency compared to NH_4^+ or NO_3^- alone, the concepts, enhancement of NH_4^+–N nutrition to the majority of crops and enhancement of NO_3^-–N nutrition to rice, have been proposed. Both measures have significantly improved plant physiological properties, N use efficiency, and crop yields.

Plant preferences to NH_4^+–N and NO_3^-–N are determined by many plant internal factors such as plant species, cultivars, and growing stages. For example, because of a lack of nitrate reductive ability, some plant species cannot efficiently use NO_3^- as their major N source. The preferences are also determined by the chemical properties of the two ions, NH_4^+ and NO_3^-, which affect N loss and N efficiency in plants, the uptake effects on absorption of other anions and cations, the balance between cations and anions in plants by uptake of NO_3^- or NH_4^+ alone, and the influence of Fe and Mn uptake or their bioavailability as well as plant tolerances to Mn and Al toxicities. External or environmental factors, such as media pH, cations and anions, NH_4^+ concentration in solution, temperature, illumination, and aeration, also have great effects on their preferences.

The toxicity produced by NH_4^+/NH_3 nutrition is an important reason for plant preferences for the two N forms. By using NH_4^+–N as the sole N source, the toxicity is often characterized by an immediate restriction in plant growth, stem lesions, leaf area decline, total biomass reduction, and finally plant death. Influence of root growth and production of short, thick, less branched and darkly colored roots is the particular feature of the toxicity. The toxicity to plants is the combinative result of internal and external factors. Different species may have different tolerances to NH_4^+ or NH_3, and thus toxicity, can appear to be different among species and even among cultivars in the same species. Carbohydrate amounts in plants substantially affect toxicity. Strong acidification of rhizosphere soil is an important environmental factor and medium acidification associated with NH_4^+ absorption has been shown to be toxic to many crop plants. The degree of the toxicity is associated with crops' sensitivity to pH. Depending

on the plant species and particular growth conditions, each of these factors may contribute to toxicity.

Two hypotheses have been put forward to explain the physiological source or the cause of the toxicity. Some scientists consider that toxicity is due mainly to free ammonia (NH_3) that affects plant growth and metabolism at low concentration levels at which NH_4^+ is found to be not harmful. The NH_3 molecules can directly penetrate the membrane and enter cells through diffusion resulting in an increase in cytoplasmic pH that inhibits Glu synthase activity as the primary cause. However, based on the fact that under conditions without the possibility to produce NH_3, the toxicity effect still exists, and it is often enhanced by water stress, NH_4^+ toxicity has been proposed. Injury of membrane and cell wall structure as well as changes of membrane enzyme activities and metabolism have been regarded as the primary causes of NH_4^+ toxicity, and the retardation of nutrient absorption, decrease of the uptake of essential cations and plant growth, disorders of NH_4^+-induced pH changes, excessive consumption of sugars as the secondary causes of the NH_4^+ toxicity.

Crops growing in solution cultures with equal amounts of NH_4^+–N and NO_3^-–N in their entire life cycle show that they did not absorb equal amounts of each N form, varying with plant species and growing stages of each crop and solution pH. The media pH affects the uptake ratio of NH_4^+–N to NO_3^-–N of wheat, and the unequal uptake of the two N forms results in significant changes in the pH in the solution. The amounts and ratios of NH_4^+–N to NO_3^-–N taken up by rice vary with growing stages. Application of NH_4^+–N at early stages and alternatively applying NH_4^+–N and NO_3^-–N at late stages may be a way for the promotion of rice growth and increasing its yield.

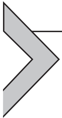
NH_4^+–N and NO_3^-–N forms affect some morphological and physiological characteristics of crop plants. Supply of NO_3^-–N can increase lateral root growth, but supply of NH_4^+–N inhibits root growth and produces abnormal growth of lateral roots. In coexistence of NH_4^+–N and NO_3^-–N, chlorophyll contents and the net photosynthetic rate are the highest, followed by NO_3^-–N, while NH_4^+–N is the lowest. NH_4^+–N, NO_3^-–N, and their mixed N source exerted a greater effect on mesophyll conductance: the highest occurs for the mixed N source, followed by NO_3^-–N and the smallest for NH_4^+–N. Some results show that on supplying NH_4^+–N, the total N, free amino acids, and amides are higher than NO_3^-–N, but others show that the two N forms have no great influence on crop N accumulation and N-containing components. Application of NO_3^-–N benefits the accumulation of sucrose and increases plant organic acids, total soluble sugar amounts, reductive sugar accumulation, structured polysaccharides contents (cellulose) in roots, stems and leaves of maize seedlings, while NH_4^+–N benefits starch accumulation and consumes large amounts of organic acids and carbohydrates and reduces sugar content. The two N forms also affect the mineral ion uptake and nutrient accumulation in plants.

The RN amount and activity are closely linked with NO_3^-–N. To a certain extent, the NRA is positively correlated to the NO_3^-–N accumulation in plants. NH_4^+–N as the sole N source inhibits NRA. Nitrate increases or maintains, whereas NH_4^+–N decreases the leaf water potential and turgor pressure.

Different N forms may produce different effects on the reactive (free) oxygen species (ROS) levels, antioxidant enzyme, and protective enzyme activities. When supplied

with NH_4^+–N and NO_3^-–N together, the activities of the superoxide dismutase (SOD) and catalase (CAT) are the highest in leaves. Nitrate addition can promote the survival and restoration of plants damaged by waterlogging; and can produce NO, a suitable amount of which increases SOD and CAT activities under a salt stress, decreases the forming-velocity and cumulative amounts of H_2O_2 and $O_2^{\cdot -}$ as well as malondialdehyde, and increases antioxidant substances.

The two N forms may affect the formation and activity of hormones and thereby may inhibit or promote cell elongation and plant growth. Compared to NH_4^+, application of NO_3^- significantly and rapidly promotes the synthesis and transport of root-originated hormones, such as cytokinin (CTK) and auxin, especially indole-3-acetic acid (IAA), and increases their activities. The IAA content of tobacco seedlings grown in 100% NO_3^-–N solution was higher than those grown in 50% NH_4^+–N + 50% NO_3^-–N solution, and the auxin concentration in the aboveground part of plants cultured by NO_3^- was higher than that for NH_4^+.



1. INTRODUCTION

Of plant nutritional elements, nitrogen (N) is the most important, essential nutrient for living organisms on earth. It is present in a number of complex organic molecules, including all amino acids, nucleic acids, proteins, and coenzymes, and plays extremely important roles in their activities (Mengel and Kirkby, 2001). As the fourth most common element in plant composition (Sun, 1980), N is outranked only by carbon (C), hydrogen (H), and oxygen (O), and it constitutes 1.5–5% of the dry weight of plants (Haynes, 1986). Among the most important organic N substances, proteins are the basic constituents of all life forms, and basic materials for protoplasm (cytoplasm) that contains 16–18% N on average. Proteins in general contain 80–85% of the total N in plants. Nucleic acids containing 15–16% N are the basic substances for plant growth and development as well as for life activity. Among the nucleic acids, the N-contained RNA and DNA are essential for genetic function. In 45–60% of the proteins, the chloroplast is the photosynthetic site, and its dry weight accounts for 20–30% of the leaf weight. Coenzymes themselves are proteins in nature, and are thus biological catalysts with a biochemical role in metabolism processes, for controlling the speed and directions of many biological and chemical reactions. In addition to alkaloids and hormones that contain N, some vitamins, such as B1, B2, B5, and PP, are also N compounds.

The complicated N compounds synthesized in plants are all initiated from simple mineral N compounds, mainly NH_4^+–N and NO_3^-–N as well as N elements. Plants can obtain these mineral N forms in different ways such as uptake by roots from the environment, especially the soil they live

in or by leaves and stems from the ambient atmosphere. The process by which plants convert the inorganic N (NO_3^-, NH_4^+, and N_2) to organic forms is the key step as well as an essential procedure in biology because animals depend on a dietary source of organic N originating from plants and microorganisms (Mengel and Kirkby, 2001). Although plants can utilize some simple organic N forms such as amino acids, amides, and amines from the soil (Shimode, 1960; Spoerl, 1948; Tsungle and Shihwei, 1942; Virtanen and Linkola, 1946; Wu and Tao, 1995; Zhang and Sun, 1984) and gaseous NH_3 from the ambient atmosphere by their canopies (leaves) (Cowling and Lockyer, 1981; Farquhar and Von Caemmerer, 1982; Hutchinson et al., 1972; Porter et al., 1972), the quantities are very limited and can even be ignored. Except for some leguminous plants that possess the capacity to fix N from the atmosphere, most plants use mineral N compounds as predominant forms. As a result, roots are the major organs for plants to take up N nutrients, and NH_4^+–N and NO_3^-–N are the main N sources for plant utilization. For this reason, these two N forms are basic substances for plant nutrition, and studies on their nutrition have been conducted for many years.

Before N was discovered as an element by Daniel Rutherford of the University of Edinburgh in 1772 (Lavoisier, 1965), Glauber (1656) (a German chemist) had already isolated saltpeter (niter heaps; KNO_3) from cattle manure (See Cheslokov et al., 1960; Glass, 1989). He correctly argued that since the cattle feed upon plants, the latter must represent the original source of this compound. Glauber demonstrated by adding saltpeter to potted plants and found that saltpeter strongly stimulated plant growth, but he mistakenly presumed that it was the sole plant nutrient (Cheslokov et al., 1960; Glass, 1989). Later on, researchers found that plants did not only absorb NO_3^- but they also absorbed NH_4^+/NH_3.

In 1804, de Saussure published his "Recherches Chimique sur la Vegetation," and established a precedent of Woodward, growing plants in solutions containing single salts and even organic compounds. As a result of these investigations, de Saussure became convinced that the normal nutrition of plants was impossible without the absorption of nitrates and other minerals from the soil, even though these elements represented only a small proportion of the plant's food. From the analyses of plant ashes, he suggested that plant composition varied with the nature of the soil and the age of plants.

Based on de Saussure's work, J. V. D. Boussingault (1834) (see McCosh, 1984) proposed the N nutrition theory, pointing out that leguminous crops could utilize N from the atmosphere to increase soil N contents, while cereals only take up soil N compounds resulting in the reduction of N contents in

soil. Later, in his book "Organic Chemistry in its Application to Agriculture and Physiology," Liebig (1841, 1865) summarized the previous results obtained by his predecessors, formulated the Law of the Minimum, which described the effect of individual nutrients on crops, downplayed the role of humus in plant nutrition, and discovered the fact that plants feed on N compounds and carbon dioxide derived from the air. He put forward a proposal that NH_4^+ or NH_4^+ salts/nitric acid were able to supply N to plants. One of his most recognized and far-reaching accomplishments was the invention of N-based fertilizers. Liebig believed that N must be supplied to plant roots in the form of NH_3, and recognized the possibility of substituting chemical fertilizers for natural (animal dung, etc.) ones. Nitrogen fertilizers are now widely used throughout the world, and their production concerns a substantial segment of the chemical industry. Although Liebig realized that plants could obtain minerals from the soil, he mistakenly concluded that the source of plant N was atmospheric NH_3. Besides, his proposals had no experimental data for support.

The midnineteenth century saw further application of de Saussure's exemplary quantitative methods for the analysis of fertilizer effects in field trials, most notably by Boussingault at Alsace and by Lawes and Gilbert in England. Liebig's notions regarding the source of N for plants were shown to be incorrect. For nonlegumes, nitrates and ammonium salts, absorbed from the soil by the roots, met the N requirements. The experiments of these workers established without doubt that "artificial" fertilizers could sustain soil fertility indefinitely. In the 1860s, von Sach and Knop reintroduced the methods of water culture, in which nutrient sources could be accurately defined. Since water culture experiments were used in plant nutrition studies, researchers such as Sachs (1882) began to use NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2CO_3$ as N sources and failed several times. Such failures were caused by the pH in the culture media that was not regulated and therefore prevented plants from growing normally and even led to their death. Based on the results obtained at that time, some conclusions were mistakenly drawn: NH_4^+/NH_3 seemed to be unsuitable as the N source for plant uptake, and only NO_3^-–N was suitable for plant needs. Later on, Schloessing and Muntz, two students of Boussingault, first defined nitrification to be a biological process, and pointed out that NH_3 could be directly used by plants without nitrification.

In identifying the function of NH_4^+–N nutrition to plants, Loo in China (Yin, 1998) and Prianishnikov (1945) in the former SSSR made an important contribution. Prianishnikov (1945), a Russian agrochemist, studied the N metabolism and the nutritional function of NO_3^-–N and NH_4^+–N and

comprehensively investigated the factors that affected NO_3^-–N and NH_4^+–N absorption. In his monograph book, "Nitrogen in Plant Life and in Soviet Union Agriculture," Prianishniko (1945) systematically discussed the absorption of NO_3^-–N and NH_4^+–N and the factors affecting their absorption. He was particularly interested in the relationship of crop species, growth stages, and sugar storage in plants with NH_4^+–N and NO_3^-–N absorption using oat (*Avena sativa*, L.), cotton (*Gossypium hirsutum* L), maize (*Zea mays* L), and yellowish pea (*Pisum sativum* L) seedlings as testing materials. Before his results were made known, scientists held the view that NH_3 was toxic to plants. However, results from Prianishnikov (1945) showed that the effect of the two N forms depended on the following conditions: if the most suitable condition was provided for each N source to play its role, principally, they could be regarded as having equal values in plant physiology. On investigation of the day and night changes of protein decomposition and the formation of asparagine, Prianishnikov (1945) found that asparagine was not only formed during the decomposition of proteins but it was also mainly formed during the decomposition of amino acids. This means that asparagine was formed by the use of the NH_3 produced during the decomposition of amino acids in plants and therefore that its formation was a secondary process. The accumulation of NH_3 was toxic to plants, while that of asparagine was not toxic. For this reason, he called aspargine "nontoxic NH_3." These results clarified the origin of NH_3 as an N source, and based on the results, he realized the importance of studying NH_4NO_3. In 1900, Prianishnikov called NH_4NO_3 "the future fertilizer," and such a conclusion was proven in later practice (Shestakov, 1954 in Russian and 1957 in Chinese).

Almost at the same time, Tsung-Lê Loo, a Chinese plant physiologist, adopted solution culture techniques to study the relationship of ammonium and nitrate salts as two N sources to plant growth, and investigated the effects of solution pH, N concentrations, cations and anions, and aeration on the absorption of the two N sources (Yin, 1998). Through his research, Loo concluded that NH_4^+–N and NO_3^-–N, the two N forms, had no nutritional differences in nature, and that some differences occurring in practice were caused by the accompanying (complimentary) characteristics of the salts. The major differences in the two N sources lie in the physiological acidity and alkalinity as well as in ion properties. From the physiological viewpoint, for example, NH_4^+ cation in ammonium salts has antagonism to divalent ions such as Ca^{2+}. Loo's experiments were conducted in a short time by using plant seedlings as testing materials. In some experiments, Loo used $NaNO_3$, NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$,

and NH_4HCO_3 to replace $Ca(NO_3)_2$ and KNO_3 in Knop solution, and used the altered solution to culture rice (*Oryza* Linn), wheat (*Triticum aestivum* L. em. Thell.), oat (*A. sativa*, L.), buckwheat (*Fagopyrum tataricum* (L.) Gaertin), faba (*Vicia faba* L), and lupine (*Lupinus* Linn). The solution was changed at certain time intervals, and the pH changes caused by the unequal absorption of ions were determined. He observed that when the N sources were NH_4NO_3, NH_4Cl, and $(NH_4)_2SO_4$, the solution, after plant culture for a certain period of time, became very acidic. However, when NH_4HCO_3 was applied, the solution became very alkaline. In contrast, by using $NaNO_3$, it was slightly alkaline, while $(NH_4)_2HPO_4$ had almost no influence on the solution pH. The difference in crop plants to tolerate pH changes had produced different effects on their growth. Increase of the buffering capacity of the cultured solution or frequent changes of solution could greatly eliminate or even remove the pH changes in solution, and it therefore avoided the ill effect of pH changes on plant growth. However, buffering the solution was not fully satisfactory if the initial solution pH was unsuitable for plant growth, and good results could not be obtained even if the solution was frequently changed. Loo (1927a) stated that if H^+ concentration increase in solution could be avoided, ammonium salts would be a good N source for higher plants and that the addition of Ca^{2+} to the solution could prevent the ill effect of NH_4^+ salts on plant growth. He also studied the effect of maize (*Z. mays* L) seedlings on the absorption of NH_4^+ and NO_3^-, and observed that in Knop solution with NH_4NO_3 as the N source, other salts had great impact on N absorption. If the concentration of other salts was very high, maize seedlings first absorbed NH_4^+, and when NH_4^+–N was taken up to a certain extent, they began to take up NO_3^-–N. In the case of high salts, the amount of NH_4^+–N uptake was far larger than with NO_3^-–N, leading to the reduction of pH; when the salt concentration was low, the NO_3^-–N uptake by plants surpassed that of NH_4^+–N, while when the salt concentration was equal to the concentration of the Knop solution, the uptake of either was equal (Loo, 1927b).

Experiments using different concentrations of NH_4NO_3 alone in solution showed that under higher concentrations of NH_4NO_3 solution ($0.00483\,mol\,L^{-1}$), maize seedlings seldom absorbed NO_3–N, while in dilute solutions ($0.00063\,mol\,L^{-1}$), amounts of NH_4^+ and NO_3 absorbed were equal. Regulating the pH in NH_4NO_3 solution or complete solution with phosphoric acid to buffer the solution to such a range between 3.8 and 8.5, NH_4^+ uptake was increased with the decrease inacidity and the increase in alkalinity, while better uptake of NO_3^- was found in slight

acid solutions. Potassium ion (K^+) in culture solution could promote the absorption of both NH_4^+ and NO_3^-, and low concentration of Ca^{2+} could promote while high concentration of Ca^{2+} could inhibit both absorption. Photosynthesis and substances stored in albumen (endosperm) and cotyledon (seminal leaf) had beneficial effects on NH_4NO_3 absorption. The absorption of maize seedlings was not only influenced by solution pH but also by uptake amounts of N. On culturing maize seedlings in solution containing NH_4NO_3 salts, due to unequal absorption of ions, the solution pH was significantly changed, and changes toward acidic or alkaline were found to be detrimental to plant growth. Based on these results, Loo (1927a, 1927b) concluded that once maize roots were made to contact with solution containing NH_4NO_3, the solution pH could be rapidly changed, and such changes influenced the growth of maize seedlings. For avoidance of the solution pH changes toward acidity or alkalinity, NH_4^+ salts were a suitable N source to plants. By combining NH_4HCO_3 and $(NH_4)_2SO_4$ in solution at a certain ratio, the pH in the nutritional solution was not greatly changed during the maize growing period, and maize could be grown well (Loo, 1929a, 1929b, 1931). Loo and Loo (1942) further investigated the effect of different organic and inorganic N sources on maize root tips, and found that when nitrate salts were used as the N source, the maize root tip was the longest, maxillary roots were the largest, and the root weight the highest. In contrast, NH_4^+ salts were an unfavorable N source to root tips. Also, maize root tips could use urea, asparagine, and albumin as the N source, but the effect of amino acids was not obvious.

The second and third decades of the twentieth century were the most productive period of time for studying NH_4^+–N and NO_3^-–N. During this period, scientists revealed NH_4^+ and NO_3^- as the major N sources for plant absorption and clarified many factors affecting their absorption. In addition to those mentioned above, other scientists such as Arnon (1937) also made great contributions. Since the 1960s, scientists continuously investigated the effect of the two N forms. Two methods were developed: one was on studying the amounts of NO_3^- and NH_4^+ uptake and their uptake effect on crop growth and the mechanism of differences of crop plant responses to the two N forms, using water culture as main method and crop seedlings as main materials (Beevers, 1976; Fried et al., 1965; Hageman, 1984; Kirkby, 1967; Mengel and Arneke, 1982; Schrader et al., 1972). The other investigated the effect of different N sources on biomass production and crop yields (Haynes, 1986; Hewitt and Smith, 1975; Warncke and Barber, 1973). Due mainly to the differences in conditions and methods used, the results obtained were quite different.

When analyzed from the plant physiology viewpoint, some researchers hold that NO_3^-–N and NH_4^+–N are equally effective to plants, and that their amounts taken up by plants are similar (Arnon, 1937; Beevers, 1976; Schrader et al., 1972). However, other researchers consider that the plant seedlings can take up more NH_4^+–N than NO_3^-–N (Coic et al., 1962, 1963; Fried et al., 1965; Yang and Sun, 1991a). From fertilizer effects, more scientists insist that the combination of the two forms should give a better result than when either is used alone. Results from Hageman (1984) and Haynes (1986) show that both NH_4^+–N and NO_3^-–N are good N sources for plants. However, in most aerobic soils, NH_4^+–N can be quickly changed into NO_3^-–N, and therefore, the latter becomes the main effective N source. Only under conditions in which nitrification is inhibited, such as soils being anaerobic or acidic, is NH_4^+–N the major N source for plants. Due to many factors influencing the effect of NH_4^+–N and NO_3^-–N, research progress is still very slow, and many problems have not yet been solved.

In this chapter, attempts have been made to critically and comprehensively summarize the progress made in the responses of crop plants to NH_4^+–N and NO_3^-–N. Most results are obtained from the literature, but some are based on our work of several years. The major focus is on two mineral forms, NH_4^+ and NO_3^-. However, since some organic N is also absorbed by higher plants, a brief introduction to the organic N nutrition is also given.

2. ORGANIC N NUTRITION FOR PLANTS

Plants can absorb either inorganic N or organic N, with inorganic N being the main N source. Great attention has been paid to the organic N function by scientists for a long time. As Hewitt (1966) pointed out that in 1868, Hampe first reported an N-source-based experiment using amino acid as the N source, and in 1898, Luts reported a vegetable-based experiment using organic and mineral N sources with sterilized culture.

Under natural conditions, most parts of the soil, particularly the soil surface layer, contains a large amount of organic matter that has high volume and different intensities in the supply of nutrients. If the NH_4^+ fixed by soil minerals is not considered, 90% N is in organic form, including large amounts of humus, nucleic acids and amino acids, amino sugars; and minor amounts of lipids, amides, vitamins, hormones, antibiotics, chlorophylls, and their derivatives (F.J. Stevenson, 1982; F.R. Stevenson, 1982; Zhu and Wen, 1992). These organic N compounds are the intermediate products originating from soil organic matter, metabolic products of microorganisms, and

plant-root-secreted ones. They are in equilibrium with minerals. Normally, these substances can be used by plants simultaneously with minerals or separately. Some of these substances may be taken up by plants earlier than minerals are, and some may be better in nutritional function than minerals are (Chapin et al., 1993; Sun and Zhang, 1996).

The difference between organic N and mineral N for plant nutrition is in the form that is taken up at that time by plants. Extensive research studies have shown that plants not only use simple organic N compounds such as amino acids (Chapin et al., 1993; Huang et al., 1965; Shimode, 1960; Spoerl, 1948; Tsungle and Shihwei, 1942; Virtanen and Linkola, 1946; Wu and Tao, 1999; Zhang and Sun, 1984), nucleotide, pyrimidine, and purine (Jin et al., 1976; Sun, 1991; Wang et al., 1975) but they also use some complicated organic N compounds such as hemoglobin (Hb) or hemoglobin, and ferrohemoglobin (HHb) (Mclaren et al., 1960). These compounds have greatly different effects on plant nutrition.

The contribution of organic N and mineral N to plant uptake depends on each concentration ratio. With the mineral N concentration in soil being $<0.1\,\mu mol\,cm^{-3}$, maize takes up >90% amino acid N of the total uptake of N (Jones and Darrah, 1993, 1994). A ^{15}N-labeled experiment shows that with equal amounts of N ($10\,mg\,N\,L^{-1}$), application of glycine (Gly) together with NH_4^+–N is better in rice dry matter production than either alone, and rice takes up 38.5% of NH_4^+–N and 35.9% Gly N; with the decrease of NH_4^+–N and increase of Gly N, maize can get the same contributive rate as NH_4^+–N can, and even higher than NH_4^+–N when Gly is applied alone (Wu, 1996).

Different plant species may have different responses to the organic N. In a water culture experiment, Chapin et al. (1993) discovered a plant (*Eriophorum vaginatum* L) grown in tundra soils in the arctic area that had a strong affinity to Gly and glutamic acid in its roots and could take up >60% amino acid N of the total N absorbed. When fed with amino acids, the biomass and N taken up by most plants were all higher than when fed with ammonium N or NO_3^-–N. However, barley, which was well grown in mineral soil, only had half of the biomass as that cultured by NH_4^+–N and NO_3^-–N. Ghosh and Burries (1950) found that DL-α-alanine, and L-asparagine had a higher effect on red clover (*Trifolium pratense* L.), but a much lower effect on tobacco compared to ammonium sulfate; Gly and L-glutamic acid had a higher nutritional effect on tomato than ammonium sulfate, but had a inhibitory effect on tobacco growth. Using double-labeled techniques, Näsholmd et al. (1998) showed that in natural ecosystems, the north forest

plants could directly absorb various amino acid N, the amounts absorbed being dependent on the species of forest plants, and generally in the range of 42–91% of the total N absorbed. Persson and Näsholm (2002) reached the same conclusions and stressed that the absorbed ratio of amino acid to inorganic N depended on the species of forest plants. Wang et al. (1997) demonstrated that winter common jujube (*Ziziphus zizyphus*) or Chinese date contained amino acids with glutamine (Gln) as the dominant one. This may be related to its responses to those N forms. Peng et al. (2006) sprayed ^{15}N-labled urea, Gly, and Glu on a 2-year pot-cultured Shandong winter date (*Zizyphus jujuba* var.*inermis* Rehd.) to study the absorption, use, and responses of the date to the three N forms. Their results showed that the three forms of N could all be absorbed by the winter date, and after absorption, they were translocated to leaves and to the top branches. However, the two amino acids were taken up much less than urea was: in leaves, Gly was only 29% and Glu 12% of urea, and in roots, 43% of urea for either Gly or Glu, respectively. When taken up by the date, the activity of glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) was increased larger by amino acids than by urea, while urea could increase soluble protein content more than the amino acids in leaves. In addition, urea could increase Gly activity while reducing nitrate reductase activity (NRA). In contrast, Gly greatly reduced NRA, whereas Glu had almost no influence on it. The possible mechanism for the reduction of NRA by Gly may relate to the amino acid being the terminal product of NO_3^- assimilation, and produce inhibitive feedback to the NO_3^- absorption. Amino acids could significantly increase the number and areas of good color, soluble solid-phase substances of date fruits, and for these effects, Glu was better than Gly was.

The effect of organic N has a close relation to its molecular structure. Spoerl (1948) found in a study of the effects of 19 amino acids on the embryo of boat orchids (*Cymbidium*) that the amino acids containing two carboxyl groups or having a long chain with more than one N atom often gave better results than the other amino acids, the mechanism of which was not known. The most natural amino acids composed of proteins in plants are of L-types, and in plants, there exist only such enzymes that can display their roles to the natural substances, and these enzymes may promote the natural L-type substances to easily participate in the metabolism (Huang et al., 1965). The organic N forms obviously influence their effects. Wu and Tao (1999) demonstrated that under a sterile solution culture condition using the ^{15}N labeling technique, Gly had more functions in promoting

dry matter production and N absorption of rice than did NH_4^+–N. At equal amounts of N levels ($4\,mg\,L^{-1}$) in sterile solutions, the effects of Gly and L-histidine were higher than that of ammonium sulfate, while L-serine, L-glutamic acid, L-arginine, DL-alanine, L-leucine. DL-aspartic acid, L-proline, L-tyrosine, L-lysine, DL-threonine, L-isoleucine, and DL-phenylalanine were lower than that of ammonium sulfate, while L-casamino acid had a certain function in the inhibition of rice growth (Sun, 1991). For an increase of the N concentration to $10\,mg\,L^{-1}$, the nutritional effect of Gly was still higher than that of ammonium sulfate (Wu and Tao, 1999). Luo et al. (1997) revealed that rice had different absorbing kinetic parameters for arginine (Arg), L-Glu, and Gly. Zhang et al. (2005) studied the effect of amino acids on strawberry by spreading ^{15}N-labeled amino acids and urea on the leaves of strawberries and found that the ^{15}N utilization efficiency was in the following order: urea > Gly > Glu. Their results also showed that tryptophan could increase the soluble solid-phase substances in strawberry fruits, total sugar contents, and vitamin C and could therefore improve the quality.

The responses of plants to the organic N compounds may also depend on the duration of applied time. Some organic N compounds may show a poor effect after application in early, short periods, but may have an effect as good as mineral N after application for a longer time. For example, for cultivation of the embryo of boat orchids with Gly for two months, the effect of Gly was lower than for ammonium sulfate, but when continued to culture to 7 months, the effect was as good as for ammonium sulfate (Spoerl, 1948).

Organic N affects plant product quality and plant metabolism. Results from Chen and Gao (2002) showed that the use of amino acids instead of NO_3^-–N could increase the soluble sugar and protein contents in leaves of loose-leaved (cabbage) pakchoi (*Brassica rapa*) and lettuce (*Lactuca sativa*) as well as the total N contents. Muller et al. (1995) showed that the uptake of amino acid N by plants could reduce the uptake of NO_3^-–N and NRA. Some research studies have shown that the presence of sugar in fruits was closely related to the accumulation and transformation of anthocyanins in the carpodermis, and one of the reasons why amino acids promote fruits having a good color might relate to the increase of soluble solid-phase substances in fruits. Wang et al. (2004b, J. Wang et al. 2006, L.J. Wang et al. 2006) believed that Glu was the precursor of 5-aminolevulinic acid (ALA) and that its promotion of apple fruits having a good color was related to ALA activity, the formation and increase of ALA promoted the formation of phytochromes, and therefore accelerated the decomposition of chlorophyll in the carpodermis, and resulted in the anthocyanins being accumulated.

Mo et al. (2003) reported that under sterile conditions, when wheat absorbed amino acid N, the activities of GOT and GPT were enhanced, the sites and amount for assimilation depending on the types and concentrations of amino acids. Aslam et al. (1996) studied barley roots and claimed that under low NO_3^- concentration ($0.1\,mmol\,L^{-1}$) conditions, supply of aspartic acid (Asp) and Glu could inhibit NRA, decrease the absorption of NO_3^-–N, while no significant effect on NRA was found when there was a high NO_3^- concentration ($10\,mmol\,L^{-1}$).

The horizontally and vertically interrelated apoplast and cytoplast are the two major pathways not only for mineral N but also for the organic N to pass through the cell wall and to arrive at the plasma membrane that may have transport proteins in relation to its structure for selection of the organic N substances. When the activity of these proteins is stimulated, their configuration may be changed, and the inner gate may be opened for information and solutes to pass across the membrane (Jiao, 1992). The selection of the substances may be mediated by adenosine triphosphatase (ATPase) activities (Wyse et al., 1985).

A number of hypotheses have been proposed to explain the mechanism of organic N transport across the membrane. In addition to endocytosis (Nishizawa and Mori, 1980; Tanchak et al., 1984), other two hypotheses are specially noted.

The solute penetration across the membrane is believed to be an active process that needs energy as the driving force. Giaquinta (1979) used *Ricinus communis* (L.) and sugar beet as materials to study the uptake and proposed the sugar/proton cotransport hypothesis. According to the hypothesis, with the proton pump driven by energy released from ATPase, H^+ formed in the cytoplasm is pumped to the outside of the plasma membrane, while some nutritional ions remain inside the cytoplasm. As a result, the H^+ concentration is higher outside the membrane, while it is lower in the cytoplasm, and therefore, an electrochemical gradient of protons is formed. Under such a circumstance, the organic N (substrate) combined with specific proteins (carriers) and proton cotransport across the membrane along the H^+ electrochemical gradient (Giaquinta, 1979). In the process, sugar and proton cotransport in the same direction, while passing through the tonoplast, sugar is antitransported with proton (antoport). This hypothesis can preliminarily explain the entire uptake of some organic N compounds by plant cells without hydrolysis before entering the cells. It has reported that in the uptake of aspartic acid, the culture solution pH was not significantly changed and that the C:N ratio was maintained constant, and no NH_3 was

released. Therefore, one can assume that this amino acid passed through the membrane in an entirly molecular form (Virtanen and Linkola, 1946). Chapin et al. (1993) also proposed that the uptake of amino acids by *E. vaginatum* (L.) was in molecular form, since the solution pH was not raised. It has been found that in rice root surface and the secreted substances from roots, there was no GOT or aspartate aminotransferase, deaminase, and histidinedecarboxylase; this further shows that amino acids are not transformed or hydrolyzed before entering the membrane (Wu and Tao, 1999; Zhang and Sun, 1984). This hypothesis can also explain the consumption of energy in the uptake process. The uptake of glutamic acid by radish, oat, barley, and potato could be inhibited by the respiration inhibitor, 2,4-dinitrophenol (Webster, 1954), showing that the uptake needs energy from respiration. Some research results have shown that the uptake of Gly, glutamic acid, and arginine by rice can be described by the Michaelis–Menten equation, and the *K*m of arginine was the smallest, followed by Gly and that of glutamic acid was the largest. The *V*max was similar to that of the three acids. This result is well in agreement with the carrier hypothesis.

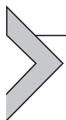
In contrast to the active uptake, a passive uptake of amino acid hypothesis is also proposed. Lemoine and Delrot (1989) found that the cytoplasm of sugar beet leaf blade can absorb sucrose similar to in diffusion without supply of energy, and if the H^+ gradient or the H^+ gradient exists together with an electric gradient, the uptake velocity of cytoplasm to sucrose was greatly accelerated, resulting in the sucrose concentration being 6- and 12-fold higher in the cytoplasm than outside the membrane. Some workers relate this uptake to ion channels (Fei, 1992; Jiao, 1992), but no evidence was given to show organic N compounds being taken up in such a way.

It is now known that organic N compounds can be transported through the plasmodesma of the symplast system from one cell to the other, horizontally and vertically, to finally reach the xylem, through which they are transported to different parts of the upper plant for use (Wu and Tao, 1996).

The organic N compounds taken up by plants can be rapidly transported and assimilated. After 5 min of application of ^{14}C-Gly to rice seedlings, some amino acids taken up could be seen on autoradiography, and after 5 h, Gly was found to be transported to leaves, and after 48 h, the uptake amount reached the maximum, and began to be transformed into other amino acids, sugars, and organic acids and participated in various metabolic systems and had a nutritional effect (Sun and Zhang, 1996).

The assimilation of organic N compounds is rather complicated, including transamination and deamination. When amino acids enter plants, they

can be assimilated through transamination (or aminotransfer) and deamination, forming other amino acids and then proteins or converted to the corresponding α-keto acids that can provide carbon skeletons for numerous carbon (C) and N metabolisms (Wang et al., 2003; Wu and Tao, 2003). Huang et al. (1965) reported that by using L-glutamine as the N source for tomatoes in vitro (detachments), a large amount of glutamic acid, alanine, γ-aminobutyric acid (GABA), and asparagine was significantly increased in addition to Gln, and the alanine contents were increased threefold and GABA increased twofold. This may be caused by the following: when Gln entered the plant it was hydrolyzed into glutamic acid and NH_3, and part of Gln formed alanine through transamination, and part formed GABA through deamination. The NH_3 produced in the process can also form other amino acids. Similar results were also obtained on using L-asparagine and aspartic acid as N sources. Using Gly and glutamic acid to culture rice, the activities of GOT, GPT, and glutamate dehydrogenase (GDH) were all higher than of ammonium sulfate, and in different organs, there existed great differences in their activities. The higher activities of GOT and GPT treated with Gly than ammonium sulfate may show that a large part of Gly taken up by rice was assimilated by roots through transamination, and the higher activity of GDH in leaf blades may show that part of Gly absorbed in roots was transported to leaves and formed energy substances, HAD(P)H and α-ketoglutaric acid, through deamination. For the same reason, the higher activity of GDH treated with ammonium sulfate than that with Gly may be caused by transformation of part of the NH_4^+–N into glutamic acid in roots and then transported to other organs (Wu and Tao, 1996).



3. NH_4^+–N AND NO_3^-–N ABSORPTION KINETICS

3.1. Concept of the Michaelis–Menten Equation

As early as 1903, A French physical chemist Henri (1903) found that enzyme reactions were initiated by a bond between the enzyme and the substrate. His work was taken up by an American biochemist Leonor Michaelis and a Canadian physician Maud Menten. In 1913, they established the simplest model describing the rate of enzymatic reactions that was one of the best-known models of enzyme kinetics (Menten and Michaelis, 1913), and it was later named the Michaelis–Menten equation; the equation is as follows:

$$V = V\text{max}\frac{C}{K\text{m} + C}$$

where C is the substrate concentration, Vmax represents the maximum rate achieved by the system at maximum (saturating) substrate concentrations, and Km is the substrate concentration when the reaction rate reaches $1/2\,V$max. The equation relates the reaction rate V to the concentration (C) of a substrate. Biochemical reactions involving a single substrate are often assumed to follow Michaelis–Menten kinetics, without regard to the model's underlying assumptions. This is a nonlinear equation. Using a graphical representation of the Lineweaver–Burk plot (or double reciprocal plot) with two reciprocals, $1/V$ and $1/C$ (Leskovac, 2003), the Michaelis–Menten equation can be changed into the Lineweaver–Burk equation as follows:

$$\frac{1}{V} = \frac{1}{V\text{max}} + \frac{K\text{m}}{V\text{max}} \cdot \frac{1}{C}$$

Also using V vs. V/C to plot, a linear relationship, $V = V\text{max} - K\text{m}\,V/C$, can be obtained for the Michaelis–Menten equation (Eisenthal and Cornish-Bowden, 1974). At present, the two transformed linear equations are widely used for data processing of absorption.

The Michaelis–Menten equation does not only describe the relation of the enzyme to the substrate concentration reaction (enzyme–substrate interaction) but it also describes a variety of biochemical situations (Abedon, 2009; Briggs and Haldane, 1925; Grima and Schnell, 2006; Jones, 2010; Keating and Quinn, 1998; Lehninger et al., 2005; Mathews et al., 1999; Schnell and Turner, 2004; Stroppolo et al., 2001; Yu and Rappaport, 1997; Zhou et al., 2008).

3.2. Utilization of the Michaelis–Menten Equation in Nutrient Uptake by Plants

Epstein and Hagen (1952) and Epstein (1953) studied the kinetics of ion uptake of crop roots and revealed that the ion uptake by plant cells and roots has a feature of saturation kinetics. This is in accordance with the assumption of control of the number of binding sites of ions (carriers or permeases), or the capacity of the proton efflux pumps in the plasma membrane, and thereby the kinetics of ion uptake is similar to the relationship between an enzyme and its substrate. Based on the fact found, they first relate the kinetic process of ion transport across the membrane of plant cells regulated by a carrier to that of the substrate catalysis regulated by the enzyme, regarding the carrier as an enzyme molecule and the ion being absorbed as the substrate for the enzyme.

As in the enzyme–substrate interaction, the ion uptake kinetics or the processes of a nutrient ion uptake by plants can also be characterized by

two kinetic parameters, *V*max and *K*m. Of these, the *V*max is a capacity factor mainly manifesting or denoting the maximal transport rate that could be reached by crops in absorption of an ion when all available carrier sites are loaded or when the number of the effective carriers reaches a maximum, that is, the maximal transport rate. Therefore, it can reflect the maximum potential of crops to absorb an ion. The magnitude of *V*max is mainly determined by the number of the effective transport carriers in the plasma membrane. The higher the *V*max, the higher the internal potential of a crop for absorbing a nutrient ion will be. The magnitude of the *K*m value, equal to the substrate ion concentration giving half the maximal transport rate, is determined by the carrier properties, and has no relation with concentration, reflecting the affinity of the ion-absorbing site of carrier (Larsson et al., 1989) for the nutrient. The reciprocal of *K*m (1/*K*m) directly reflects the affinity of an ion-absorbing site in root plasma membranes to a given nutrient or ion. The smaller the *K*m value for absorbing an ion of a crop, the larger the affinity of the crop to the ion, and therefore the stronger the absorbing capacity will be.

For the equation describing ion uptake, *V* and *V*max are the ion absorption velocity, and thus have the same unit, but it varies for different authors. Some use the ion-absorbed amount (nmol or pmol) per unit time (s) per unit root surface area (cm^2 or m^2) (Barber, 1984a); some use the ion-absorbed amount (nmol) per unit time (s) per unit root length (cm or m) (Barber, 1984a; Mengel and Kirkby, 1987), while others use the ion-absorbed amount (μmol) per unit time (h or min or s) per unit root weight (g) (fresh root weight in most case) that has been widely used in China (Ni, 1982; Yang and Sun, 1991b). C is the concentration of a nutrient and *K*m relates to the concentration, and thus both have the same unit $mmol\,L^{-1}$.

The two kinetics parameters of root absorbing kinetics (*K*m and *V*max) can to a certain extent evaluate the ability of a crop's roots to absorb an ion and can express the efficiency of root absorption of the given ions. If a crop has a high absorption velocity and high affinity to an ion, the crop may take up high amounts of the ion and have a high efficiency. Otherwise, the amount may be reversed. When the concentration is low, the uptake is often well described by the Michaelis–Menten kinetics. However, when the nutrient concentrations are high, the accompanying anion or cation has an effect on the uptake rate of the cation and vice versa.

The work of Epstein and Hagen (1952) opened up a new possibility for evaluation of ion absorption kinetics, and a large number of research studies followed thereafter. Claassen and Barber (1974) established the ion-depleted

technique, and this technology has provided an effective way for measuring the characteristics of nutrient uptake by roots in the study of nutrient absorption kinetics (Xie, 1988). In studying NH_4^+–N and NO_3^-–N absorption kinetics, the two parameters have been widely used for identification of the N nutrition difference of different crop species or different cultivars of the same species (Ni, 1982; Yang and Sun, 1991b) and also for the evaluation of different genotype adaptations to soil nutrition conditions (Ni, 1982). The two parameters can also reveal the maximum velocity and affinity of crops to NH_4^+–N and NO_3^-–N, and crop preference to the two ions. This does not only provide effective measures for the increase in N use efficiency but also for breeding and selection of crop cultivars that have a high N-absorbing and utilizing efficiency.

Some workers consider that NO_3^- uptake by roots is an active process that can be generally described by the Michaelis–Menten equation (Haynes, 1986). Thus, in studies of N absorption kinetics of crops, more attention has been given to NO_3^-–N, and the two parameters have been substantially adopted to characterize the NO_3^--absorbing efficiency, the difference of plant species or cultivars in absorption of NO_3^-, and even the difference of N nutrition of plant species and cultivars, as well as the ability of various cultivars to adapt the nutritional conditions of media (Nielsen and Schjoerring, 1998). Some scientists postulate that there are three absorbing systems of NO_3^-–N that are significantly different in plant roots, and in their absorption kinetics (Crawford, 1995; Morgan et al., 1985). However, such a viewpoint has not been accepted.

Due to the reciprocal of *K*m showing the root absorbing affinity to ions, and *V*max, the ion amount combined with the absorbing sites, such genotypes with a lower *K*m value and a higher *V*max value can absorb nutrients in a wide range of ion concentrations. Hybrid rice and nonhybrid rice have no great difference in the *V*max for absorption of NO_3^- and NH_4^+, but the hybrid rice has significantly lower *K*m than the nonhybrid rice (Yang, 1988). Herbage or pasture and crop variety grown in unfertile soils have a low *K*m and *V*max for absorbing NO_3^-–N (Huffaker and Rains, 1978). Ni et al. (1988) found that there were great differences existing for the absorption kinetics of maize cultivars to NO_3^-–N and believed that the kinetic parameters would be helpful for the evaluation of the ability of maize cultivars for absorbing soil NO_3^-–N. However, studies from Gary and Peter (1986) show that there was no obvious difference for *K*m among cultivars of maize, and thereby, it was impossible to depend on *K*m or *V*max as an index for the evaluation of plant N-absorbing capacity.

Several workers have attempted to relate the short-term kinetic parameters of the NO_3^- uptake to the adaptation of plant species to nutrient-rich and nutrient-poor habitats. Huffaker and Rains (1978) found that three grass species having low, slightly high, and high *K*m values for NO_3^- uptake agreed well with the corresponding nutrient-poor, intermediate, and high environments. However, Van de Dijk et al. (1982) found no significant differences in the *K*m values for NO_3^- uptake (range 10–20 μM) between five grassland species characteristic of soils of widely different nutrient status. On the other hand, the *V*max values for the NO_3^- uptake by the species were related to the nutrient status of their natural habitats. Cacco et al. (1980) used *V*max and *K*m to evaluate the adaptability of various cultivars to the absorption of NO_3^- process by plants. They classified the absorption process of NO_3^- into four types based on the combinations of *V*max and *K*m: high *V*max and high *K*m genotype plants that adapt to high nutrient concentration and are suitable to be used in sufficient supply of water and fertilizer; high *V*max and low *K*m genotype plants that adapt to wide-range nutritional conditions and are regarded as an ideal model; low *V*max and low *K*m genotype plants that adapt to unfertile areas and low nutrient concentration; and low *V*max with high *K*m genotype plants that are unfavorable to any nutritional concentration conditions and find it difficult to live without any advantages.

Cultivars of the same plant species differ greatly in ion uptake. Experiments using spinach, garden sass, pakchoi, tomato, lettuce, rice, wheat, maize, tobacco, cotton, and others show that all the crops in the uptake of NH_4^+ and NO_3^- have significant genotype differences (Barker et al., 1974a, 1974b; Chen et al., 2004, 2005a, 2005b; Dai et al., 2001; Dong et al., 2006; H.R. Dong et al., 2004; Feng et al., 2003; Guo et al., 1999b; He et al., 1999; C.D. Li et al., 2003; C.F. Li et al., 2003; Luo et al., 2005; Sun et al., 2005; Walter et al., 1996; J. Wang et al. 2006; L.J. Wang et al. 2006; Van Beusichem et al., 1988). Addition of NH_4^+ to the environment can affect the parameters of the absorption kinetics of NO_3^-. Youngdahl et al. (1982) found that NH_4^+ could affect the *V*max for plant uptake of NO_3^- while having little effect on *K*m. This means that NH_4^+ mainly affects the effective number of carriers for absorption of NO_3^- with a much lesser effect on the affinity of the absorbing sites in root cells to NO_3^-–N. The transport velocity of carriers to ions is restricted by at least two factors: number of carriers and the environment for carriers in membranes (Larsson et al., 1989). In another words, the effect of NH_4^+ on NO_3^- absorption is perhaps influenced by two ways: affecting the expression of the gene controlling the synthesis

of NO_3^- carrier proteins, and thereby reducing the total amount of carrier proteins in the plasma membrane and also affecting the ambient environment of the carrier-existed plasma membrane. For example, changes of membrane potential (Schubert and Yan, 1997), the polarized degree of the membrane (Crawford and Glass, 1998), and membrane structure (Colmer and Bloom, 1998) can decrease the transport velocity of carriers to ions. Furthermore, the absorption of NH_4^+ across the membrane has led to the alteration of the cytoplasm pH (Kosegarten et al., 1999), and this may alter the metabolism process and the NH_4^+ assimilated products, which may further inhibit NO_3^- absorption as a feedback mechanism or inhibit the reduction of NO_3^- in plants.

Some determinative results of the root absorption kinetics in wheat, rice, maize, barley, and ryegrass are not consistent (Barber, 1984a; Ni, 1982; Wang et al., 1990; Yang, 1988; Yang and Sun, 1991b; Youngdahl et al., 1982). According to numerous research studies, the *V*max and *K*m for plant uptake of NH_4^+–N and NO_3^-–N vary with crop species, cultivars, plant age, N concentration in solution, temperature, and light intensity.

Ni (1982) and Wang et al. (1990) showed that under low concentration (0.05–2.0 mmol L^{-1}) and high concentration (5–50 mmol L^{-1}) conditions, the uptake velocity for NH_4^+–N and NO_3^-–N was in agreement with the dual mechanism proposed by Epstein (1972) and Hodges (1973) for describing ion absorption. However, in the low concentration group, *V*max and *K*m were all lower, while in the high concentration group, both were higher. Such differences may be caused by absorbing sites that are different (Epstein, 1972).

3.3. Responses of Crop Seedlings to NH_4^+–N and NO_3^-–N in Relation to Kinetic Parameters

For demonstration of the NH_4^+–N and NO_3^-–N uptake amounts in relation to their uptake kinetic parameters, we use a typical study as an example. He et al. (1999) studied the effects of different ratios of NH_4^+–N to NO_3^-–N on the growth of six crops including spring wheat, maize, proso (*Panicum miliaceum*), buckwheat, tomato (*Solanum tuberosum* L.), and pakchoi, using Prianishnikov's nutritional culture solution in which NH_4^+ and NO_3^- were present in equal N amounts. Five ratios of NH_4^+–N to NO_3^-–N (100:0, 75:25. 50:50, 25:75, and 0:100 mg L^{-1} N) were designed, and Arnon's micronutrient mixed solution was added to form a complete nutrient solution with the pH at 6.5. Depending on the plant species, 7- to 14-day-aged seedlings of crops, with almost the same fresh weight per plant

for each species, were transplanted to a 1-L volume pot in which solutions with different ratios of the two N forms were added and the pH was regulated. Plants were grown under normal light, temperature, and humidity conditions. Wheat, maize, and buckwheat were cultivated for two weeks and proso, tomato, and pakchoi for three weeks. After harvest, the fresh weights (including roots), fresh root weight, and total dry weight were measured as criteria for evaluation of the plant preference to the two N forms.

Results show that the six crops have different responses to the two N forms as shown in their biomass (Table 5.1). When grown for 2 weeks, the fresh root weight, total fresh weight, and total dry weight of wheat increased with the increase of NO_3^-–N ratio while it decreased with the increase of NH_4^+–N ratio in solution. Although no significant difference was found between the three combinations of the two N forms, wheat biomass was significantly lower on supplying NH_4^+–N than on supplying NO_3^-–N as the sole N source. When supplied with NO_3^-–N alone (ratio of 0:100), wheat biomass was significantly higher than ratios of 50:50 and 75:25. The magnitude of wheat biomass ranked in the following order: 100:0 < 25:75 < 50:50 < 75:25 < 100:0. Obviously, NO_3^-–N is the best N source followed by the ratio of 25:75 of NH_4^+–N to NO_3^-–N.

Maize dry biomass weight was the lowest in NH_4^+–N solution and the highest in the three combinative solutions of the two N forms. The three N combinative solutions were not significantly different in maize biomass. Although NO_3^-–N was better for biomass weight than for NH_4^+–N, neither source seemed to be fully beneficial for root growth as shown in their lowest fresh root weight. Neither source alone was as good as a combined supply of the two. With an increase of the NH_4^+–N ratio in the solution, fresh root weight increased, but total fresh and dry weights were not significantly different. As a whole, at the seedling stage, the best combinations of NH_4^+–N to NO_3^-–N were 75:25 and 50:50.

The biomass of proso grown for three weeks was significantly higher in solutions with two N forms than that with one form alone, and the lowest total fresh weight and fresh root weight amounts were found with NH_4^+–N followed by NO_3^-–N. However, the biomass amounts for different NH_4^+–N and NO_3^-–N combinations were not significantly different. Thereby, proso also needs the two N forms together, and suitable ratio of NH_4^+–N to NO_3^-–N covers a wide range from 75:25 to 25:75.

The biomass for buckwheat grown for two weeks varied in a similar pattern to that of wheat. The total fresh and dry weights and fresh root weight were increased with an increase in the NO_3^-–N ratio, whereas it declined

Table 5.1 Effects of Different Ratios of NH_4^+–N to NO_3^-–N on the Growth of six Crops Seedlings

Crop	NH_4^+–N: NO_3–N	Biomass			Relative biomass
		Total fresh weight	Root fresh Weight	Total dry weight	
Water culture for 2 weeks (g/10 plants)					
Wheat	100:0	3.30 c	1.03 c	0.375 b	70.4
	75:25	3.91 b	1.34 b	0.382 b	80.9
	50:50	4.05 b	1.43 b	0.383 b	83.6
	25:75	4.25 a	0.39 b	0.393 ab	90.6
	0:100	4.72 a	1.81 a	0.445 a	100.0
Water culture for 2 weeks (g/5 plants)					
Maize	100:0	16.06 d	6.85 d	1.51 b	96.4
	75:25	23.71 a	9.80 a	1.77 a	117.4
	50:50	23.64 b	8.93 b	1.78 a	112.3
	25:75	22.57 ab	8.02 c	1.74 a	106.4
	0:100	21.10b	6.90 d	1.57 b	100.0
Water culture for 3 weeks (g/10 plants)					
Proso	100:0	17.30 c	4.90 c	1.64 b	76.6
	75:25	28.10 a	6.90 b	2.94 a	124.3
	50:50	32.70 a	9.50 a	2.72 a	139.5
	25:75	30.30 a	9.80 a	2.50 a	164.0
	0:100	23.60 b	6.80 b	1.93 b	100.0
Water culture for 2 weeks (g/10 plants)					
Buckwheat	100:0	3.00 c	0.31 c	0.20 c	68.1
	75:25	4.30 b	0.39 c	0.23 b	83.6
	50:50	4.23 b	0.44 b	0.23 b	84.5
	25:75	4.61 b	0.53 a	0.25 b	94.2
	0:100	5.19 a	0.53 a	0.28 a	100.0
Water culture for 3 weeks (g/50 plants)					
Tomato	100:0	3.06 c	0.33 b	0.19 c	104.0
	75:25	5.40 a	0.46 a	0.31 a	164.8
	50:50	4.29 b	0.33 b	0.21 b	120.2
	25:75	3.49 c	0.28 c	0.20 c	104.4
	0:100	3.30 c	0.29 c	0.18 c	100.0
Water culture for 3 weeks (g/10 plants)					
Pakchoi	100:0	3.32 c	0.28 d		40.6
	75:25	4.02 c	0.43 c		56.5
	50:50	6.36 b	0.48 bc		73.2
	25:75	6.85 b	0.55 ab		81.5
	0:100	9.22 a	0.62 a		100.0

He et al. (1996).

with increases of NH_4^+–N. Application of NO_3^-–N alone gave the best results, while application of NH_4^+–N alone the poorest. The total fresh and dry weights for the three NH_4^+–N and NO_3^-–N combinations had no significant difference. The relative biomass followed the order of 0:100 < 25:75 < 5 0:50 < 75:25 < 100:0. Obviously, the buckwheat seedlings preferred NO_3^-–N, and the suitable ratios of NH_4^+–N to NO_3^-–N were 0:100 and 25:75.

Neither NH_4^+–N nor NO_3^-–N had a great difference in the total fresh and dry biomass for tomato grown for three weeks, but NH_4^+–N alone was better for root growth than NO_3^-–N was. In the combinative solutions, the total fresh and dry weights were significantly increased with NH_4^+–N ratio while they were decreased with an increase in NO_3^-–N. The total biomass on average was in the following order: 75:25 > 50:50 > 100:0 > 25:75 > 0: 100, and the 75:25 ratio was the best while 0:100 the poorest. Obviously, tomato preferred NH_4^+–N and the better ratio of NH_4^+–N to NO_3^-–N was 75:25.

The total fresh biomass and root weights of pakchoi grown for three weeks increased with an increase in the NO_3^-–N ratio, and decreased with an increase in NH_4^+–N. The highest biomass was obtained by supply of NO_3^-–N as the sole N source while the lowest by NH_4^+–N alone, the total fresh weight of the former being three times that of the latter and the fresh root weight of the former being more than two times of the latter. The ratios of 25:75 and 50:50 were not significantly different in either total or root fresh weights, nor were those of 75:25 and 100:0 although the 0:100 ratio (NO_3^-–N) was significantly higher than the ratios of 25:75 and 50:50, and the of 25:75 and 50:50 ratios were significantly higher than the ratios of 75:25 and 100:0 in both total and root fresh weights. The fresh biomass of different treatments was as follows: 100:0 < 25:75 < 50:50 < 75: 25 < 100:0. It is clear that supply of NO_3^-–N alone was the best while the supply of NH_4^+–N alone the poorest of all. Previous studies (Barker et al., 1974a, 1974b; Wang and Li, 1996, 2004) show that application of NO_3^-–N alone can easily accumulate NO_3^-–N in plants, and it affects the quality of vegetables. For increasing the yield as well as improving the quality, NO_3^-–N should be applied as the main N source in combination with a small amount of NH_4^+–N.

Obviously, different ratios of NH_4^+–N to NO_3^-–N had different effects on the biomass of the six crop seedlings. As the ratio of NO_3^-–N in the solution increased, the biomass for wheat, buckwheat, and pakchoi increased, showing a preference for NO_3^-–N. For the two combinative N forms, biomass amounts of maize, proso, and tomato were larger than for

Table 5.2 The Absorption *V*max and *K*m Values of 6 Crops to NH_4^+–N and NO_3^-–N

Crop	Age of seedlings (days)	*V*max [μmol g^{-1} (FW·root) h]		*K*m (mmol L^{-1})	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-
Wheat	8	6.67	7.42	0.29	0.27
Corn	15	4.42	3.81	0.34	0.35
Proso	14	7.22	7.29	0.22	0.23
Buckwheat	8	6.35	6.16	0.27	0.21
Tomato	14	8.01	1.89	0.11	0.19
Pakchoi	9	11.56	17.29	0.30	0.13

He et al. (1996).

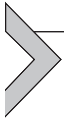
either alone. In the three combinative solutions, the biomass of maize and tomato was increased with an NH_4^+–N increase, showing preference to NH_4^+–N. However, the preference to NH_4^+–N or NO_3^-–N was not the sole characteristic. Although crops show a certain preference to NH_4^+–N or NO_3^-–N, application of the sole N form they prefer may not be optimal for fertilization, and addition of other N sources to the nutrition solution that contains the N form that plants prefer can often give a more significant result in increasing crop production. The optimum ratio of NH_4^+–N to NO_3^-–N for crop seedlings is different for different crops: the ratio for wheat, buckwheat, and palchoi varies from 0:100 to 25:75; for maize, between 75:25 and 50:50; for tomato, 75:25; for proso, from 75:25 to 25:75.

These results of crop responses to the two N forms were obtained under water culture conditions. Crop responses to the two N forms in solution must be extremely different from the responses in fields that can be interrupted by external N sources. In fertilization practice, soil, climate, irrigation, and agricultural measures should be comprehensively considered to form a rational ratio of NH_4^+–N to NO_3^-–N.

The results obtained by He et al. (1999) also show (Table 5.2) that the *K*m of wheat seedlings to NH_4^+–N and to NO_3^-–N was close to each other, but the *V*max for the uptake of NO_3^-–N was larger than that for NH_4^+–N. Therefore, at the seedling stage, wheat absorbed more NO_3^-–N than NH_4^+–N. The root affinity of maize seedlings to NH_4^+–N and NO_3^-–N was also close to each other, but the uptake velocity of NH_4^+–N was larger than of NO_3^-–N, and thus took up more NH_4^+–N. The root *V*max and *K*m of proso seedlings for both NH_4^+–N and NO_3^-–N were similar, and therefore, the uptake amount of either was not greatly different. Buckwheat seedlings had a higher *V*max to NH_4^+–N than to NO_3^-–N, but the affinity

was reversed. Due to the interaction of the two factors, buckwheat took up a similar amount of NH_4^+–N to NO_3^-–N. Tomato seedlings had a far higher velocity and affinity to NH_4^+–N than to NO_3^-–N, and thereby preferred NH_4^+–N. Pakchoi seedlings had a higher *V*max and affinity to NO_3^-–N than to NH_4^+–N, and therefore, the main N form taken up by this crop was NO_3^-–N.

From the results, we can see that the preferences of the six crop seedlings to either NH_4^+–N or NO_3^-–N were in agreement with their absorption kinetic parameters. However, the absorption kinetics parameters were not fully in agreement with the entire preference of the crops in their whole growing period, the difference depending on crops. Of the six cops, the seedling *V*max and *K*m of wheat and pakchoi, were in agreement with those in the entire growth period while for other crops, this was not the case. Due to such differences at different stages, the results obtained at the seedling stage cannot be extended to other stages or to the whole period of a plant life.



4. NH_4^+–N AND NO_3^-–N MOVEMENT TO THE ROOT SURFACE, UPTAKE, ASSIMILATION, AND TRANSPORT IN PLANTS

Most plant species need much N during the vegetative stage, whereas in the generative phase, the N uptake rates by roots are low and the retranslocation of organic N is high. Both NH_4^+ and NO_3^- are the available N sources for the direct uptake by plants. However, the two N sources have some differences in absorption, transport, assimilation and plant growth, and physiological effects (Cechin and Fumis, 2004).

4.1. Movement of NH_4^+–N and NO_3^-–N to Plant Roots

The two N sources cannot be absorbed until they move toward root surface areas. Their migration can occur in three ways: root interception, mass flow, and diffusion (Barber, 1962, 1966). All the ways are directly or indirectly related to soil water. Sufficient supply of water can make plants easily extend their roots to a depth, and be therefore beneficial to plant root interception of nutrients; the diffusion coefficient of nutrients is higher in water than in any other medium, and thus, sufficient water benefits diffusion. During the plant growth period, roots continuously absorb water and nutrients from soil, and thus in the rhizosphere zone form a depletion area of water and nutrients, resulting in the nutrient

difference between the rhizosphere and the bulk soil. This promotes water movement from high potential areas slightly far or away from the roots to low potential areas near the roots until equilibrium, and nutrients dissolved in water will migrate similarly. Such a manner does not only accelerate nutrient diffusion but it will also promote nutrient movement with water. The process of nutrient movements driven by water is called mass flow, and it leads to a decline of nutrient concentration gradients (Barber et al., 1963). As positively charged, NH_4^+–N can be adsorbed by soil colloids, a small amount exists in soil solution. In contrast, NO_3^-–N is negatively charged and mainly exists in soil solution. For this reason, the two forms of N are different in migration. In addition to the direct uptake by interception, NH_4^+ ion movement toward root surface area mainly depends on diffusion. It has been proved that NH_4^+ diffuses around a point to the outside in a ball-like shape, and its concentration is inversely proportional to the distance diffused. If the soil water content is approaching saturation and water contains a high concentration of NH_4^+ ions after N fertilization in particular, NH_4^+–N may penetrate deeply to a low layer of the soil by mass flow as occurred in rice cultivation (Chen, 1957; Ji and Wang, 1978; Sun, 1987; Xuan, 1985). In contrast, NO_3^-–N movement mainly depends on mass flow. Barber (1979a, 1979b; 1984a, 1984b) and Barber et al. (1963) discussed several times the solute movement, and pointed out the role of mass flow in N migration. Mengel et al. (1969) reported that in dryland areas, the contribution of interception, mass flow, and diffusion on wheat N uptake was 82%, 7%, and 11%, respectively. An experiment from Renger et al. (1981) showed that the contribution of NO_3^-–N through mass flow to the total uptake N of sugar beet, spring wheat, and spring barley was 100%, 40%, and 110%, respectively. We studied the movement of NH_4^+–N and NO_3^-–N under natural and root-restricted conditions, and found that the NH_4^+ concentration was not different between rhizosphere and bulk soil, while the NO_3^-–N concentration was much lower in rhizospheres than in bulk soil (Li et al., 2009). Even if NO_3^-–N can move with water, in dryland areas with water deficits, mass flow cannot fully supply N to rhizospheres that are deficient in N due to crop absorption. In general, the diffusion velocity of NH_4^+–N is lower than that of crop uptake, and therefore, the rhizosphere at the top horizon is often deficient in this N form. The deficient phenomenon in rhizospheres for NO_3^-–N also occurs, but when the NO_3^-–N concentration is high, and water is abundant, enrichment of NO_3^-–N in rhizospheres also happens occasionally.

4.2. NH_4^+–N and NO_3^-–N Uptake

Higher plants generally acquire the bulk of their nutrient elements from the environment surrounding the root. Nutrients destined for use by the shoots must therefore first move through the root tissue before entering the xylem and being translocated to the shoots (Luttge and Higinbotham, 1979).

In general, roots can be subdivided into the epidermis, cortex, endodermis, and stele to from a cross-sectional viewpoint. The stele contains the xylem, through which ions are translocated to shoots, and the phloem, through which photosynthate is supplied from the shoots to the roots. Ions entering the cell of the epidermis are first taken up and move through the epidermis, cortex, endodermis, and stele and get emptied into the xylem from which they are translocated to other parts of the plant (Barber, 1984a). The movement occurs from cell to cell.

Each cell, the smallest viable unit of living matter, consists of a cell wall, plasma membrane, and cytoplasm or cell contents (Barber, 1984a). The cell wall structure is made up of pectic substances, cellulose, and hemicellulose. Cellulose tends to aggregate to form a chain-like structure known as microfibrils. Intermicrofibrillar spaces allow the entry of water, air, and solute particles into the cell wall (Mengel and Kirkby, 2001), and thus, the cell wall is permeable to all these materials. The outer epidermal wall may have a cuticle layer (Barber, 1984a). The plasma membrane or plasmalemma inside the cell wall is the membrane boundary between the cytoplasm (protoplasm) and the cell wall, being a barrier to passive movement of ions into and out of the cell. Inside the plasma membrane is the cytoplasm or the cytosol (the cytoplasm without the organelles) in which the most important organelles in the cell are located, including the nucleus, chloroplast (in leaves) or plastids (in roots), and mitochondria. The light energy conversion and CO_2 assimilation take place in chloroplasts and enzymes controlling the various steps of the tricarboxylic acid (TCA) cycle, respiration, and fatty acid metabolism are present in the mitochondria. The membrane between the cytoplasm and vacuole is the tonoplast that separates the cytoplasm from the vacuole.

Cells are connected by the intercellular bridges (plasmodesmeta) and form a continuum of cytoplasm from one cell to the cytoplasm of adjacent cells. The continuous plasmatic connection occurring in the cells of a tissue is called the symplasm or symplast, and the movement of ions through the plasmadesma connection is called the symplast (symport) pathway. Ions diffuse into the cell walls of the epidermal cell and then move freely through the cytoplasm from the epidermal cells, the cortex, and endodermal cells to the cells of the stele. Absorption of ions across the plasmalemma of root

cells is generally believed to be an active process that often overcomes an unfavorable electrochemical gradient through expenditure of energy. It had been assumed that the active uptake might be accompanied by carriers or channels that were visualized as protein units in the plasmalemma (Haynes, 1986), and now, it has been proven that the active uptake is accompanied by transporters (Hawkesford et al., 2011).

In addition to the symplast, within a plant, ions may also move passively into the continuum of nonliving cell wall material, the free diffusion space outside the plasma membrane, of the epidermal and cortex cells. This is a continuous system of water and air filled spaces of cell wall in close contact with the soil medium (Haynes, 1986). The area in which ions can freely move is called free space or apoplast, and this process is known as apoplastic transport. Structurally, the apoplast is formed by the continuum of cell walls of adjacent cells as well as the extracellular spaces, forming a tissue level compartment comparable to the symplast. However, in addition to the influence of air spaces between plant cells and the cuticula of the plant, this transport mechanism is interrupted by the hydrophobic bands of suberin deposited around the radical walls of the endodermal cells, known as the Casperian strip, a barrier to ion movement from the cell wall space of the cortex into the cell wall space of the stele. This essentially restricts apoplastic movement from the free space of the cortex to the free space of the stele. However, the active transport through symplasts is associated with the ions from the apoplast, and the ions transported by the apoplast are absorbed across the plasmalemma of the cortical and endodermal cells, and thus enter and then get discharged into the symplast. Solutes entering the vascular tissue of roots possessing an intact endodermis must therefore do so by first being absorbed across the plasmalemma of the epidermal, cortical, or endodermal cells and then moving through the symplast. The apoplastic route facilitates the transport of water and solutes across a tissue or organ (Campbell and Reece, 2002) and is important for all the plant's interaction with its environment. In the roots, ions diffuse into the apoplast of the epidermis before being taken up into the symplast by specific ion channels and being pulled by the plant's transpiration stream. Similarly, all gaseous molecules emitted and received by plants such as plant hormones and other pheromones must pass through the apoplast. The apoplast is also a site for cell-to-cell communication (Felle et al., 2005).

Once NH_4^+–N and NO_3^-–N arrive at the root surface, their uptake by plant roots occurs. On the one hand, both ions can directly penetrate the cell wall of the epidermis and enter the apoplast by diffusion or mass flow,

and on the other hand, the two ions enter the symplast by active transport through the plasma membrane barrier. In the latter way, NH_4^+ and NO_3^- can move radically across the different cell types of roots and pass the endodermal Casparian trip. For transporting to the shoots, NO_3^- is loaded from the symplast of the stele cell into the apoplast of the xylem for long-distance transport via the transpiration stream. Several NO_3^- transporters have been found to exert a specific role in controlling the NO_3^- distribution within the shoots such as AtNRT1.8 in roots likely to mediate unloading of NO_3^- from the xylem for uptake into leaf cells in the shoots (Li et al., 2010) and AtNRT1.7 to transport NO_3^- across the plasma membrane into the phloem of older leaves (Fan et al., 2009). This allows NO_3^- remobilization from older (source) leaves to N-demanding (sink) tissues.

When taken up by plant roots, NH_4^+ is assimilated or stored in vacuoles in the roots or is transported to aerial parts. In the past, it was believed to be impossible for NH_4^+ to be used for long-distance transport within the plant. However, the fact that NH_4^+ concentration of the xylem can be in the milimolar range (Finnemann and Schjoerring, 1999; Yuan et al., 2007) suggests that NH_4^+ could be transported from the roots to the shoots. NH_4^+ is generated by photorespiration in chloroplasts of illuminated leaf cells, lignin biosynthesis, amino acid catabolism, and protein breakdown in senescing tissue and is also supplied from the nodules following nitrogen fixation in legumes. Although the transporters involved in xylem loading and unloading in the shoot, and in distribution of NH_4^+ within the shoot and within plant cells are largely unknown at present, such transport is obviously important throughout the plant to move NH_4^+ from the source to the sink.

NH_4^+ and NO_3^- can enter the cytoplasm (efflux) through the apoplast or from the cytoplasm into the apoplast and then the external solution (efflux). Within the cytoplasm of any individual cell, there exist many organelles surrounded by membranes or barriers separating them from the cytoplasm. For entrance into the cell, NH_4^+ and NO_3^- must first overcome the barrier of the plasma membrane into the cytoplasm and then pass through other membranes, especially the tonoplast, for assimilation. In their development process, plants have evolved many mechanisms for the two N forms to pass through the biomembranes.

4.2.1. NO_3^- Uptake

NO_3^-, as a dominant form of mineral N generally present in higher concentration (1–5 mM) than NH_4^+ (20–30 μM) in the soil solution of most

agricultural soils (Owen and Jones, 2001) and more mobile in the soil than NH_4^+ and therefore more available to plants (Miller and Cramer, 2004), is the form usually taken up in the greatest amounts by crops, readily mobile in the xylem and is stored in vacuoles of roots, shoots, and storage organs.

An important way for NO_3^- ions penetrating the membrane along the electrochemical gradient (Barkla and Pantoja, 1996) is through channels that may be membrane pores allowing the facilitated diffusion of water molecules (Chrispeels and Maurel, 1994) or a complex formed by the solute with a cation (Barkla and Pantoja, 1996). The latter mechanism called antiport or cotransport is a secondary active transport because only by combining the solute with a cation to form a positively charged complex is the transport along the electrochemical gradient achieved (Bush et al., 1993). Proton as a cation plays a major role in the proton cotransport with one or several protons being combined with the solute (an ion, a sugar, or an amino acid). Since these anions carry negative charges, they require an excess of protons to form a positively charged complex. The required H^+ is provided by the proton pump in the plasmalemma and the tonoplast. Plasmalemma ATPase activity is also of importance for the retrieval of anions and metabolism. As a consequence of the negatively charged cytosol, anions may diffuse through channels out of the cytosol along the electrochemical gradient. These anions may be reabsorbed by proton cotransport, the so-called retrieval mechanism, and there is a lack of H^+ in the outer solution for the retrieval process (Matzke and Mengel, 1993). Blocking the ATPase by vanadate results in a major release of anions.

As an anion, the uptake of NO_3^- is thus believed to be mainly transported across membranes by the proton–anion cotransport (symport) or exactly by the H^+/NO_3^- cotransport (Clarkson et al., 1993; Ullrich, 1992; Ullrich and Novacky, 1981, 1990). In such cotransport, the needed energy is mainly supplied from the H^+ concentration gradient. Using protons as the driving force, NO_3^- is cotransported together with H^+ across the membrane against the steep electrical (potential difference) and chemical (pH difference) gradient. The entrance of NO_3^- can maintain the balance of cell electrochemical potential. Due to cotransport of H^+ and NO_3^-, with an increase of NO_3^-–N uptake, the membrane is temporarily depolarized (Zhou et al., 1998). Evidence for the H^+/NO_3^- cotransport in the plasma membrane of root cells has been identified (McClure et al., 1990a, 1990b). More and more workers are in agreement with the H^+ pump mechanism of NO_3^- or the mechanism of the cotransport of NO_3^- and H^+, and consider that at least two-thirds of NO_3^- (2:1 in ratio) is transported in this way. It

has now been proved that the OH^- flow from cells (efflux) has no obvious relation to the NO_3^-–N absorption. During the process, the H^+ is pumped out of the cell by the plasmalemma proton, and then, it is transported back (recycled) together with NO_3^- into the cytosol to neutralize the negative charge of the cytosol (Ullrich, 1992; Ullrich and Novacky, 1981). Hence, NO_3^- uptake is associated with a pH increase in the outer media. The NO_3^- uptake process is also regulated by other factors such as the internal N nutritional status of the plant (Crawford and Glass, 1998; Imsande and Touraine, 1994; Siddiqi et al., 1989). In the uptake process (influx), the NO_3^-–N can also be released to the outside of the cell (efflux), and thus, the net uptake of NO_3^- is a result of its influx and efflux (Oscarson and Larsson, 1986).

In contrast to NH_4^+, NO_3^- can be transported at high rates into the upper parts of the plant and can be stored at high concentrations in the vacuole (McIntyre, 1997). The uptake rate of NO_3^- is dependent on the energy status of plants (Rufty et al., 1989). If the plasmalemma ATPase is inhibited due to the lack of protons required for NO_3^- cotransport, the efflux is particularly high (Matzke and Mengel, 1993). The NO_3^- uptake rates depend also on the activity of nitrate reductase (NR) (Aslam et al., 1992). Without supply of NO_3^- as an N source, the seedlings had a very low NRA, and a high-affinity uptake system with very low Vmax for NO_3^- was operating while those with the induced NR had a high-affinity transporter system for NO_3^- with a Km of 0.7 mmol m^{-3} being present and the Vmax being about four times higher than that in the noninduced plants. All the results show that the plant processing sensitive N uptake systems may be adjusted to N demand and environmental conditions.

Besides the proton–anion cotransport system, the NO_3^- transport through the membrane may be also mediated by transport proteins or carriers that may occur in biological membranes. These carriers can transport an ion from one side of the membrane to the other side by means of carrier molecules that are able to diffuse through the membrane. Although the transport proteins for inorganic anion in higher plants seldom exist, some workers assume that the carrier protein for transferring NO_3^- may exist in plasma membranes of cells. Results using NO_3^- labeled with an extremely short-half-life isotope ^{13}N ($t_{1/2} = 10$ s) to investigate the absorption of NO_3^- have proven that the transport proteins had a saturation property (Lee and Clarkson, 1986; Lee and Drew, 1989). Studies on the net absorption kinetics of NO_3^-–N show that in the NO_3^- transport proteins, there exist at least two types of saturable component and one type of

nonsaturable component. One of the saturated components is characterized by high affinity (low in *K*m) and low absorption velocity (low in *V*max), while the other by low affinity (high in *K*m) and high *V*max. As a result, the absorption kinetics curves for NO_3^- often occur in double-saturated forms. Studies on the absorption kinetics of some higher plants such as maize, barley (*Hordeum vulgare* L), and tobacco (*Nicotiana* L.) also show that there are two NO_3^- transfer systems: a high-affinity transport system (*K*m 5–300 μM) that ensues plant growth under low NO_3^- (10 μM) nutritional media and a low-affinity transport system (*K*m < 0.5 mM) that can absorb high amounts of NO_3^-–N and ensures plant growth under high NO_3^- concentration nutritional media. However, some workers demonstrate that the specific ion channels in plant cell membranes are more important for ion transport across the plasma membrane than carriers and a NO_3^- channel is postulated at the tonoplast (Tyerman, 1992). Ion channels are unique among transport proteins in their ability to regulate ion flux subject to the physical–chemical environment of the channel protein (Blatt and Thiel, 1993). These channels allow rapidly passive permeation (uniport) of ions through membranes. Open channels catalyze the permeation of 10^6–10^8 ions s^{-1}, which is at least three (Tester, 1990) to even five (Bentrup, 1989) orders of magnitude faster than carrier-mediated transport ions. Hedrich and Schroeder (1989) reported that carriers transport 10^4–10^5 ions carrier^{-1} s^{-1}, whereas channels transport a passage of >10^6 ions s^{-1}. However, ion channels are closed most of the time, and their number seems to be rather small.

Recently, it has been found that the uptake of NO_3^- into plant roots is mediated by transport proteins located in the plasma membrane of the epidermal and cortical root cells. Several physiological transport systems mediate the uptake of NO_3^- with different affinities. In higher plants, two types of transporters are involved in root NO_3^- uptake, belonging to the NRT1 (Ho et al., 2009; Liu and Tsay, 2003; Tsay et al., 2007) or NRT2 (Okamoto et al., 2003) families that transport NO_3^- across the plasma membrane in symport with protons (Forde, 2002), which in turn requires the expenditure of the H^+-adenosine triphosphate (ATP) by the H^+-ATPase for proton extrusion to maintain the proton gradient over the plasma membrane. Molecular genetics in Arabidopsis have shown that although at least four NO_3^- transporters are involved in the NO_3^- uptake into roots, there is no simple one-to-one relationship between these genes and the physiological uptake systems (Tsay et al., 2007). Irrespective of the type of NO_3^- transporters, the inward transport of NO_3^- across the plasma membrane occurs against a steep electrochemical potential gradient because the negatively

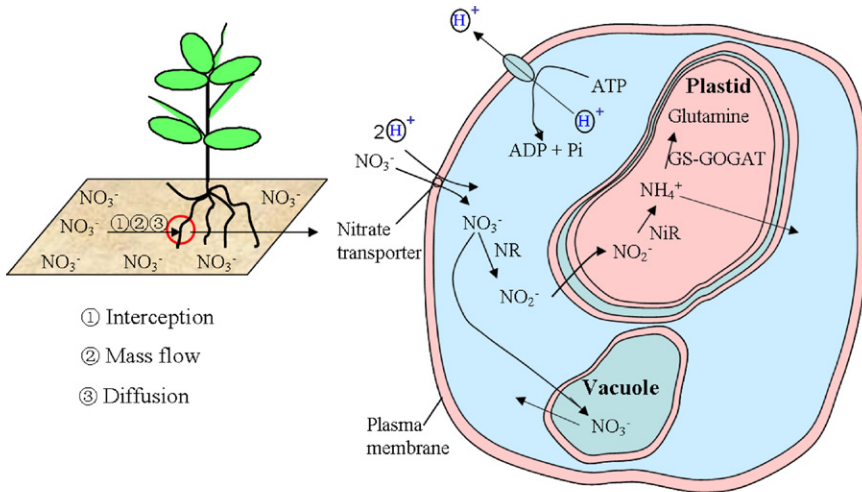


Figure 5.1 NO_3^- uptake by plant root cells. For color version of this figure, the reader is referred to the online version of this book.

charged NO_3^- ion has to overcome both the negative plasma-membrane potential as well as an uphill concentration gradient. NO_3^- influx therefore requires metabolic energy (Fig. 5.1).

In addition to the influx of NO_3^- into epidermal and cortical root cells that is essential to plant growth, efflux of NO_3^- back into soil solution can also occur, mainly due to excess N supply (Aslam et al., 1996; Kronzucker et al., 1999). Such a process may play a role in the sensing of NO_3^- availability by providing a dynamic and flexible regulation of cytosolic NO_3^- homeostasis (Miller and Smith, 2008) and NO_3^-–N net uptake (influx minus efflux). At low external NO_3^-, the chemical gradient and the electrical gradient favor passive efflux of NO_3^- from the cytosol across the plasma membrane (Miller and Smith, 1996). Although this gradient is present for many hours after N deprivation, the efflux nevertheless decreases and ceases after a few hours, suggesting that in the absence of external NO_3^-, NO_3^- efflux is downregulated (Van der Leij et al., 1998).

4.2.2. NH_4^+/NH_3 Uptake

NH_4^+ is in equilibrium with NH_3 that is a weak base with a pK_a of 0.92. In most soils, the pH is considerably lower than this pK_a, and NH_3 concentrations are usually very low. Thus, the NH_4^+ is the main form taken up by roots, and protein-mediated influx of NH_3 into roots plays a minor role (Loqué and von Wirén, 2004).

NH_4^+ uptake through the plasma membrane has been assumed to occur in three ways, either active or passive, or both.

The passive uptake way was proposed much earlier. Nye and Tinker (1977) revealed that the uptake of NH_4^+ by plants occurred in two stages: at the initial stage conducting in the free space of roots, the NH_4^+ uptake was a passive exchange process that was not affected by low temperature and metabolism inhibitors, such as KCN, while at the second stage, it was an active absorption process susceptible to low temperature and metabolism inhibitors. As a passive way, NH_4^+ ion passing through the membrane is believed to be in either NH_4^+ or NH_3 form based on the fact that when potassium and NH_4^+ ions are supplied simultaneously, potassium uptake is reduced significantly, while NH_4^+ is almost not affected. This shows that the competition for uptake sites between NH_4^+ and potassium has no effect on NH_4^+ uptake. Since NH_3 can rapidly get protonated to form NH_4^+ and NH_4^+ can get deprotonated to produce NH_3 (Schuurkes et al., 1986; Wieder et al., 1990), an explanation is thus proposed that when the absorbing site (carrier or channel) for the NH_4^+ uptake across the membrane is inhibited, a large amount of NH_3 is produced by the deprotonation of NH_4^+ and the NH_3 produced can pass through membranes by rapid diffusion, and thus, the total uptake amount of NH_4^+ is not significantly changed and is affected by the competition of absorbing sites (Kleiner, 1981; Roberts et al., 1982). Experimental results from Heber et al. (1974) show that roots absorb NH_3, while others show that roots absorb NH_4^+ in exchange with H^+. Mengel et al. (1976) found that under reductive conditions, the uptake of NH_4^+ by rice plants was not affected by potassium concentrations in media, and there was a strict equivalent relationship between the absorption of NH_4^+ and the release of H^+. These results support the view that under high pH and reductive conditions, NH_4^+ is probably absorbed in the form of NH_3. Hasson and Van Hai (1976) hold the view that NH_4^+ absorption is similar to other cations with one valence, especially potassium ion, and both have the same carrier, and thereby the two types of cations show a complete effect. However, Zsoldos and Haunold (1982) observed that the effect of low pH on K^+ absorption was different from low pH on NH_4^+. Mengel and Kirkby (2001) point out that NH_4^+ is deprotonated in the membrane and gets transformed to NH_3 that is then transferred through the membrane. Like the exchange absorption of NH_4^+ with H^+, deprotonation can also make the media acidified. Their experiments evidenced that the uptake moles of NH_4^+–N by maize seedlings was almost the same as of protons released. Plants exude protons for the uptake of NH_4^+–N and absorb H^+

(not exude OH^-or HCO_3^- as the early hypothesis) for the uptake of NO_3^- anions, and thus, the pH in the soil around roots (rhizosphere) decreases or increases. In general, the NH_4^+–N concentration in soil solution varies only in the range of 10–50 μmol L^{-1} (Novoa and Loomis, 1981), and the *K*m (in range of 10–70 μmol L^{-1}) and *V*max for NH_4^+ uptake vary greatly for different crop plants (Fried et al., 1965). Excessive accumulation of NH_4^+ in the cytosol may lead to necrosis of plant tissue. The NH_4^+ concentration in the cytosol is a function of influx into cells and efflux of NH_4^+ to the apoplast, depending on the compartmentation of NH_4^+ into vacuole and NH_4^+ assimilation in the cytoplasm or plastids (Nielsen and Schjoerring, 1998).

The uptake of ^{15}N-labeled NH_4^+ was associated with an efflux of nonlabeled NH_4^+ originating from a soluble N fraction in the roots. The efflux rate was about 10% of the influx rate (Mengel and Kirkby, 2001). In NH_4^+-sensitive barley plants, high NH_4^+ influx is counteracted by an active efflux of NH_4^+ back to the soil (Britto et al., 2001; Kronzucker et al., 2001). This results in an apparently futile cycling of NH_4^+ ions across the plasma membrane (Britto et al., 2001). From the efflux of NH_4^+ from cytosol, another passive uptake way of NH_4^+ across the plasma membrane is proposed in relation to facilitated diffusion. The so-called facilitated diffusion is in fact via channels that simply allow the transport of solute across the membrane along the electrochemical gradient (Barkla and Pantoja, 1996). Since NH_4^+ can lead to a sharp depolarization of the plasma membrane, its uptake is believed to be brought about by facilitated diffusion (Ullrich 1992). Due to very low concentration of NH_4^+ in the cytosol, the electrochemical gradient favors facilitated diffusion of NH_4^+ across the plasmalemma. Since NH_4^+ resembles K^+ in ionic radius and size of hydration shell (Howitt and Udvardi, 2000), NH_4^+ ions may be able to permeate the plasma membrane through K^+ channels (Mengel and Kirkby, 2001; ten Hoopen et al., 2010; White 1996), analogous to that of K^+ brought about by the electropotential difference and cation selective channels with the difference being that K^+ may accumulate in the cytosol to high levels in the range of 100 mol m^{-3}, whereas the entered NH_4^+–N is quickly assimilated (Kosegarten et al., 1997) by the glutamine (z-) oxoglutarate aminotransferase (GOGAT) pathway and the cytosol NH_4^+ concentration is in the range of 2–30 mol m^{-3} (Feng et al., 1999). The low K^+ concentrations often observed in NH_4^+-fed plants may lead to the upregulation of K^+ channels to improve the plant K^+ uptake, potentially resulting in further NH_4^+ influx through them (ten Hoopen et al., 2010). Also, a similar passive entry of NH_3 is found across tonoplast. It has been proven that the cytosolic NH_3 is passively transported

across the tonoplast where the acidic environment traps NH_3 as traps NH_4^+, and NH_3 and water have similar sizes and polarity, allowing NH_3 to permeate through water channels in some cases. Accordingly, members of the tonoplast intrinsic proteins have been shown to play a role in NH_3 import into the vacuole (Jones and Takemoto, 2004; Loqué et al., 2005). In vacuoles, the NH_4^+ concentration of nonstressed plants ranges from 2 to 45 mM (Miller et al., 2001).

In addition to the passive uptake, NH_4^+ has also been believed to be taken up by an active way. Since NH_4^+ absorption can be retarded by metabolism inhibitors, the uptake is thought to be against electrochemical potential gradient, and therefore, the process is an active absorption from thermodynamic viewpoint. This absorption process depends on ATPase activity in membrane. Of the active absorptions, the H^+/NH_4^+ cotransport has been particularly noted (Schachtman and Schroeder, 1994). Different from the H^+/NO_3^- cotransport system, with NH_4^+ nutrition recycling H^+ back into the cytosol is restricted and the H^+ pumped out of the cell remains mainly outside. Hence, the pH is depressed as is obviously evidenced when plants are grown in solution culture without pH buffering capacity. Such forms of transport may be of importance when the NH_4^+ concentration in soil solution is extremely low. In a study of the NH_4^+ uptake of rice seedlings in relation to the NH_4^+ concentration of the nutrient medium, Wang et al. (1994) found that the uptake was biphasic with a curve up to 1 mol $NH_4^+ m^{-3}$ followed by a linear relationship at higher concentrations. This result is in good agreement with the concept that in the lower concentration range, NH_4^+ uptake is mediated by a specific transporter system having a high affinity, whereas at higher concentration, its uptake mainly occurs by a facilitated diffusion through NH_4^+-specific channels (Mengel and Kirkby, 2001). Kronzucker et al. (1996) reported analogous results in an NH_4^+ uptake system in roots of *Picea glauca* operating at 2.5–20 mmol m^{-3} in the uptake solution with a *K*m of 20–40 mol m^{-3}. The low-affinity transporter was linear over a concentration range of 20–40 mmol m^{-3}. Plants with a low NH_4^+ supply before the uptake experiments showed a higher NH_4^+ uptake than did plants that were well supplied with NH_4^+(Wang et al., 1994). Gazzarrini et al. (1999) found three constitutive NH_4^+ transporters (AMT) in roots of *Arabidopsis thaliana* inducible by N starvation, showing that plants are equipped with efficient NH_4^+ uptake systems for quick adaptation to N supply. More recently, workers propose that the major entry pathway for root uptake of NH_4^+ (Loqué and von Wirén, 2004) is carried out by transport systems. There are two transport systems for the NH_4^+ uptake by

roots: the high-affinity transport system and low-affinity transport system. The former is a saturable NH_4^+ uptake system that operates at NH_4^+ concentration <0.5 mM (Glass and Siddiqi, 1995; Kronzucker et al., 1996; Wang et al., 1993), while the latter dominates at NH_4^+ concentration >0.5 mM and is responsible for NH_4^+ uptake at high concentrations that may result in toxicity. All plants express a nonsaturable, low-affinity influx system (Kronzucker et al., 1996; Wang et al., 1993), which is at least partially protein mediated. Molecular biological studies have shown that NH_4^+ transport through the plasma membrane by the high-affinity system that is interposed by AMT is the major pathway for NH_4^+ uptake (Gazzarrini et al., 1999; Kaiser et al., 2002; Loqué et al., 2006; Yuan et al., 2007). Throughout the plant, NH_4^+ transport is to a very large extent carried out by members of the NH_4^+ transporter family (von Wirén and Merrick, 2004), especially the AMT1 family that transports NH_4^+ via NH_4^+ uniport or NH_3/H^+ symport. Some NH_4^+ transport proteins have been cloned, and it has been proven that NH_4^+ transport across the membrane is in a one-way inward movement (direction) (Howitt and Udvardi, 2000; Loqué and von Wirén, 2004; Ludewig et al., 2002) (Fig. 5.2).

NH_4^+ uptake depends much on the carbohydrates in the roots (Kirkby and Hughes, 1970; Michael et al., 1970). Similar to the diurnal pattern of NO_3^- uptake, NH_4^+ uptake also increases during the day with a maximum at the end of the light period after which the uptake decreases (Gazzarrini et al., 1999; Glass et al., 2002). This diurnal pattern shows that N uptake is

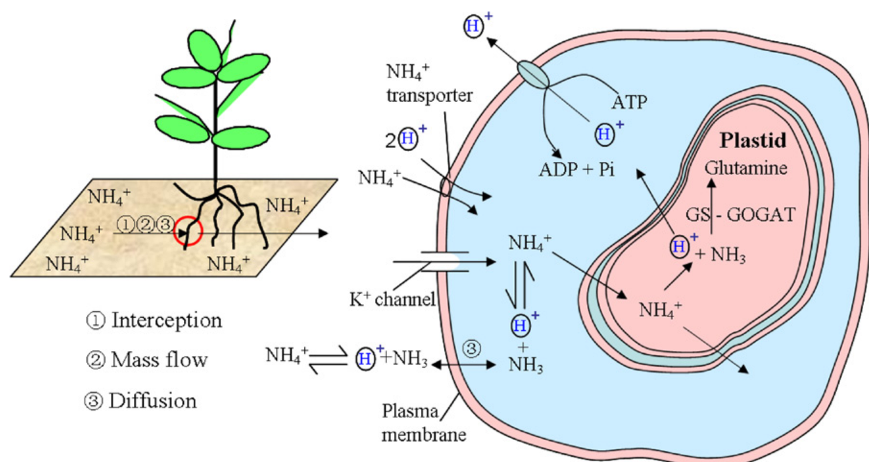


Figure 5.2 NH_4^+ uptake by root cells. For color version of this figure, the reader is referred to the online version of this book.

regulated by C supply (T.Y. Liu et al., 2009; X.Z. Liu et al., 2009). Indeed, external supply of photoassimilates leads to NH_4^+ influx in the dark via upregulation of transcription of the AtAMT1 genes as well as of the NO_3^- transporters AtNRT2.1 (Lejay et al., 2003). Feng et al. (1999) found a diurnal carbohydrate concentration change in maize roots that declined during the night while it increased during the day. This finding is in agreement with the difference of NH_4^+ uptake amount during daytime and night time. Assimilation of NH_4^+ in the roots requires carbohydrates translocated from shoots to provide the C skeleton and the energy (ATP and NADPH) for the NH_4^+ assimilation process. When there was insufficient supply of carbohydrates due to high temperature and reparative decomposition of carbohydrates, plants of strawberries died (Ganmore-Neumann and Kafkafi, 1983).

The uptake of NO_3^- and NH_4^+ can, however, induce large changes in the pH of the rhizosphere of up to 2 pH units higher or lower than the pH of the bulk soil (Marschner et al., 1991; Smiley 1974). An important difference between NO_3^- and NH_4^+ uptake is in their sensitivity to pH. NH_4^+ uptake takes place best in a neutral medium, and it is reduced as the pH decreases. The converse is true for NO_3 absorption, a more rapid uptake occurs at low pH because more protons are available for the proton cotransport of NO_3^-. At low pH levels in the NH_4^+ nutrient solution, root growth is often depressed. Experiments with faba beans from Yan et al. (1999) show that root depression comes about because H^+ ions rediffuse into the cytosol to stress the cytosolic H^+ buffer system by decreasing the malate concentration, which is needed for the maintenance of H^+. Plant roots may adapt to low pH conditions by alteration of *K*m and *V*max of the plasmalemma ATPase (Yan et al., 1999). NO_3^- uptake can be depressed by NH_4^+ as shown in young wheat plants (Blondal and Blane, 1973; Minotti et al., 1969a) and rice (Kronzucker et al., 1999) while the NH_4^+ uptake by rice is not affected by NO_3^- (Mengel and Viro, 1978).

4.3. NO_3^- Reduction

When NO_3^- enters root cells of plants, a large part is reduced and transformed into amino acids and proteins in the root system, part may transport to the shoots through hadrommestone and may be then reduced there, and/or part may temporarily get stored in vacuoles in roots, stems, and leaves.

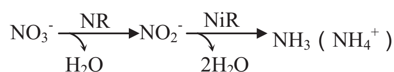
4.3.1. Process of NO_3^- Reduction

Although NO_3^-–N, as a main mineral N form, can be directly absorbed by plants, it is not the form that can be directly used by plants. In any case,

the NO_3^-–N entering plants cannot be directly transformed into organic N compounds prior to assimilation until being reduced into NH_3 that can participate in the metabolism/synthetic process and play its nutritional role. In order for NO_3^-–N to be incorporated into organic structures, NO_3^- must be reduced into NH_3.

The process of NO_3^-–N reduction to NH_3 occurs in two ways and is catalyzed by enzymes. The first step takes place in the cytoplasm and NO_3^-–N there is catalyzed by NR into nitrous acid (HNO_2) with two electrons transferring from NO_3^-–N to nitrite N and HNO_2 must pass through the membrane in molecular form. In the NO_3^- reduction process into NO_2^-, majority of plants use nicotinamide adenine dinucleotide (NAD) as electron pair donor; some may use nicotinamide adenine dinucleotide phosphate (NADP) as electron donors. Ullrich (1992) proposed the H^+/NO_3^- cotransport mechanism. That is, plant uptake of 1 mol NO_3^- into the cytoplasm will cotransport 2 mol H^+-ATP mainly provided by the respiration of mitochondria for the maintenance of an H^+ gradient.

The second step occurs in chloroplasts (shoots) or proplastids (roots), and HNO_2 is catalyzed by nitrite reductase (NiR) into NH_3 with 6 electrons transferring from nitrite to NH_3. NiR is encoded in higher plants by a single gene (Kant et al., 2011) that is localized in leaf chloroplasts and root proplastids and other nongreen tissues. In green leaves, the electron donor is the reduced ferredoxin that is generated by photosystem 1 during photosynthetic electron transport in the light. Electrons from the reduced ferredoxin are passed to nitrite via a ferredoxin-binding domain, an iron–sulfur cluster, and a siroheme cofactor bound to the NiR enzyme. In root plastids, the reduced ferredoxin is generated via nicotinamide adenine dinucleotide phosphate (NADPH or NADH) in the pentose phosphate pathway coupled with ferredoxin-$NADP^+$ reductase (Bowsher et al., 2007). The reaction is shown below:



In chloroplasts, the NH_3 formed is synthesized into amino acids by direct use of the energy (ATP) produced in photosynthetic reaction and the assimilatory power (NADPH or NADH), and therefore, this process is called NO_3^- photosynthesis, that is, photosynthetic nitrogen metabolism (Wang et al., 2003). For this reason, the importance of reduction and assimilation of NO_3^- for the life of plants is similar to that of the reduction and assimilation of CO_2 in photosynthesis (Hawkesford et al., 2011).

In C4 plants, NR and NiR are located in the mesophyll cells, while these enzymes are absent in the bundle sheath cells. Thus, the mesophyll cells use light energy for NO_3^- reduction and assimilation and bundle sheath cells for CO_2 reduction. This is most probably the cause for higher photosynthetic N use efficiency in C4 compared with C3 plants (Sage et al., 1987). Due to the particular CO_2 concentration mechanism in the bundle sheath cells, C4 plants require less RuBP carboxylase (Rubisco; with N < 10% plus 2–5% N for phosphoenolpyruvate (PEP) carboxylase) than C3 plants with N in Rubisco accounting for 20–30% of the total leaf N (Sage et al., 1987).

With supply of NO_3^- to plants, little accumulation of NO_2^- occurs, indicating that the NO_3^- reduction to NO_2^- is the rate-limiting step for NO_3^- assimilation, and the NO_3^- reduction is mainly controlled by the absorbing velocity, while the role of NRA (Wilkison and Crawford, 1993) or reductive capacity (Warner and Huffaker, 1989) is in the second position. In the second step, nitrite produced may be possibly accumulated. To prevent accumulation of nitrite that is toxic to plant cells, the NRA is regulated by several mechanisms (Lillo, 2008) such as inactivation of NR by protein binding due to the protein kinase, the inhibition of the inactivation by triose and hexose phosphates, and restoration of the enzyme activity by dephosphorylation by a phosphatase that prevents protein binding and inhibition. During short-term light–dark transitions, posttranslational inhibition of NR occurs within a few minutes preventing accumulation of nitrite (Lea et al., 2006).

The two enzymes for NO_3^- reduction are very complicated in their structure. The key enzyme for NO_3^- reduction is NR, known as a cytosolic induced-enzyme, and its half life is only a few hours (Beevers and Hageman, 1983). Plants may have no such enzyme when they have no opportunity to absorb NO_3^-–N. However, with the supply of NO_3^-–N for a few hours such an enzyme will be induced in plants (Oaks et al., 1972), and the expression of the NR genes is strongly and rapidly induced within a few hours after the addition of NO_3^- (Patterson et al., 2010). Additionally, the concentration of NR protein is increased by light, sucrose, and cytokinin (CTK), whereas Glu, a primary product of N assimilation, represses nitrate reductase (Krapp et al., 1998). The reduction of NO_3^- needs energy, and it is said that the assimilation of 1 mol NO_3–N, even in the lowest way of energy consumption, 305 light quanta are needed (Raven, 1985), while the further assimilation of NH_4^+ needs C skeletons (α-ketoglutaric acid) that mainly originates from photosynthetic carbon metabolism (Aslam and Huffaker, 1984). Therefore, the metabolism of NO_3^-–N is closely associated

with photosynthetic carbon metabolism (Stitt and Krapp, 1999). The relation of NR to Fe, Mo, N, light, and other factors have been extensively investigated (Ai et al., 2002; H. Chen et al., 2000; X.P. Chen et al., 2000; H.B. Du et al., 2001; Feng et al., 1986; Gao et al., 1990; Lin et al., 1986; Liu and Yang, 2000; Men and Li, 2005; Yu et al., 1997).

For reduction and assimilation of NO_3^-, large amounts of energy and C skeletons are required compared to NH_4^+–N. The uptake processes are particularly cost when carried out in roots (Gavrichkova and Kuzyakov, 2010; Schilling et al., 2006). Considering this aspect, supply of NH_4^+–N to plants is more economic and can obtain higher biomass production compared to that of NO_3^-–N. Using ATP as indicator, a reduction of 1 mol of NO_3^- consumes 15 mol of ATP, and synthesis of NH_4^+–N consumes 5 mol of ATP (Salsac et al., 1987). As an example, NO_3^-–N absorbed by barley is reduced mainly in roots: the consumed energy for absorption (5%), reduction (15%), and assimilation of the reduced NH_4^+–N (3%) reaches 23% of the energy produced by root respiration, while the supply of NH_4^+–N only needs 14% of the energy (Bloom et al., 1992). In contrast, for NO_3^- reduction in leaves, reducing equivalents can be directly provided by photosystem 1 and ATP from photophosphorylation. Under low-light conditions or in fruiting plants (Hucklesby and Blanke, 1992), this may lead to a competition between CO_2 and NO_3^- reduction. However, under high-light conditions and excessive light absorption (photoinhibition and photooxidation), NO_3^- reduction in leaves may not only use energy reserves but also alleviate high-light stress. Plants, except those that reduce and assimilate NO_3^-–N in roots, have taken different measures to save carbon skeletons such as (1) not reducing the absorbed NO_3^-–N to NH_4^+ in root cells but transporting to green parts in which reduction of NO_3^- and synthesis of amino acids are conducted; (2) storing the NO_3^-–N in vacuoles temporarily without immediate assimilation; (3) transferring to the xylem in NO_3^-–N form. NO_3^- is easy to move in the xylem and transport from the catheter of the xylem with transpiration stream to upper plant parts and different organs of plants. In green tissues, NO_3^- nutrition does not consume more carbohydrates stored than NH_4^+–N nutrition, and in the presence of light, NO_3^-–N assimilation is faster and can prevent the toxicity induced by the accumulation of NH_4^+ due to deficiency of carbohydrates.

In most plant species, both roots and shoots are capable of NO_3^- reduction, and roots may reduce between 5% and 95% of NO_3^- taken up. The reductive proportion or ratios of NO_3^-–N in roots, stems, and leaves depend on plant species, the storage of carbohydrates in plants and

NRA, as well as external concentration of NO_3^-. In general, the arboreal (wood) plants such as those grown in Australian open forest plants (Stewart et al., 1990) or in cerrado and forest communities in Brazil (Stewart et al., 1992) assimilate NO_3^- mainly in leucoplasts (leucoplastid) of epidermis and cortex cells, while herbal plants mainly in chloroplasts of leaf blades. For temperate perennial species and temperate annual legumes, most of the NO_3^- is reduced in the roots at relatively-low external concentrations, but tropical and subtopical annual and perennial species tend to reduce a large proportion of NO_3^- in shoots even at low external supply, whereas no change occurs in the proportion between root and shoot reduction when the external concentration is increased. With high NO_3^- availability, shoots seem to be the predominant site of NO_3^- reduction in both fast- and slow-growing grass species (Scheurwater et al., 2002). The preferential sites, roots, or shoots, of NO_3^- reduction may have an important economy of plants, and probably have ecological consequences for the adaptation of plants to low-light and high-light conditions. Our results show that the reduction rate of NO_3^-–N in roots or shoots of maize also depend on the plant growing stage (Table 5.3). At early stages, maize took up a small amount of NO_3^-–N, and most of it was reduced into NH_4^+–N in roots, while amino acid was undetected; at the middle stage, maize vigorously grew and absorbed a large amount of NO_3^-–N, in addition to reduction of part of it, a large part still existed in NO_3^-–N form with the formation of large amounts of amino acid; at later stages, the accumulative NO_3^-–N in roots was declined, but still had a certain amount.

In plant species in which most or all NO_3^- assimilation occurs in the shoots, organic acid anions are synthesized in the cytoplasm and stored in the vacuole to maintain both cation–anion balance and intracellular pH. The maintenance of pH is required because NO_3^- reduction consumes two protons per NO_3^- reduced. This may lead to osmotic problems if NO_3^- reduction proceeds after the termination of leaf cell expansion (Britto and Kronzucker, 2005; Raven and Smith, 1976). Several mechanisms exist for the removal of excess osmotic solutes from the shoot tissue.

In addition to plants themselves, environmental conditions have great impact on the absorption of NO_3^-–N. Of these, the NO_3^-–N amounts taken up by plants, NO_3^-–N distribution in different parts, and NO_3^- concentration in media (Marschner, 1986; Mckee, 1962; Mengel, 1983; Reddy and Reddy, 1993) are most important. If the uptake is small, the proportion reduced in roots will be large, and that transported to shoots will be small. In contrast, if the uptake is large, the proportion reduced in roots will be small,

Table 5.3 NH_4^+–N, NO_3^-–N, and Amino Acid N in Maize Sap at Different Growing Stages with 5 N Rates Under Plastic Sheet Mulched and Nonmulched Conditions

Time (date/month)	N form	N rate (kg ha^{-1}) 0	30	60	90	120	Mean
N content (mg plant^{-1} day^{-1}) without plastic sheet mulching							
23/5	NO_3^-–N	0.04	0.17	0.29	0.09	0.32	0.18
	NH_4^+–N	0.27	0.44	0.44	0.47	0.47	0.50
6/6	NO_3^-–N	0.04	0.10	0.00	0.51	0.21	0.23
	NH_4^+–N	0.14	0.33	0.07	1.19	0.07	0.38
18/6	NO_3^-–N	0.04	1,32	0.04	0.07	0.10	0.51
	NH_4^+–N	1.83	2.10	0.98	1.70	1.32	1.59
	AA–N	2.60	2.68	1.88	2.66	2.44	2.41
26/6	NO_3^-–N	0.21	0.18	1.73	1.31	1.03	0.89
	NH_4^+–N	0.30	0.27	0.54	0.54	0.49	0.53
	AA–N	0.49	0.42	0.55	0.55	0.95	0.64
7/7	NO_3^-–N	0.01	0.02	0.02	0.01	0.01	0.01
	NH_4^+–N	0.16	1.11	0.29	0.26	0.30	0.43
	AA–N	0.12	0.18	0.11	0.03	0.10	0.11
18/7	NO_3^-N	0.01	2.03	4.94	6.60	8.25	4.84
	NH_4^+–N	3.02	5.28	8.92	12.61	14.92	9.00
	AA–N	1.73	2.98	2.66	8.30	6.30	3.24
N content (mg plant^{-1} day^{-1}) with plastic sheet mulching							
23/5	NO_3^-–N	0.55	1.29	0.85	0.95	1.46	1.02
	HH_4^+–N	1.60	1.35	0.60	0.67	0.70	0.98
6/6	NO_3^-–N	0.89	2.29	1.68	2.60	2.58	2.01
	NH_4^+–N	1.02	2.02	2.37	1.69	1.14	1.65
18/6	NO_3^-–N	3.80	2.93	2.06	2.52	2.94	2.30
	NH_4^+–N	5.86	5.11	4.36	14.60	7.96	6.96
	AA–N	4.38	4.19	4.00	5.28	4.26	4.13
26/6	NO_3^-–N	0.11	0.81	2.76	2.95	4.83	2.30
	NH_4^+–N	0.83	2.25	3.04	3.88	5.39	2.54
	AA–N	1.00	1.11	1.15	1.05	1.45	1.15
7/7	NO_3^-–N	0.01	0.04	0.01	0.01	0.08	0.03
	HN_4^+–N	0.25	0.30	0.21	0.26	0.29	0.27
	AA–N	0.07	0.11	0.06	0.08	0.09	0.08
18/7	NO_3^-–N	0.02	0.18	0.40	5.86	6.86	2.65
	NH_4^+–N	2.21	2.06	3.12	4.10	10.85	4.47
	AA–N	1.05	1.14	2.98	3.42	5.04	2.73

Li et al. (1993) (unpublished data).

and a large portion will be transferred to leaves and get reduced there. The solution concentration directly affects the uptake amount, and the temperature affects the metabolism intensity. The uptake of NO_3^- is greatly affected by the pH of the medium. Under the same conditions, low pH is beneficial to NO_3^- uptake, while high pH is beneficial to NH_4^+–N uptake. When the pH increases in soil, NO_3^-–N uptake is reduced. There are two possible reasons for the reduction: competition of high OH^- concentration to NO_3^- and the influence of cell pH increase. When NO_3^- absorbed is assimilated in plants, the cell pH increases, and alkalinity is increased, which needs organic acids formed in cells to neutralize while pH increase influences the formation of organic acids, and absorption of NO_3^-–N by plants. In addition, the coexisting or complementary ions, such as Ca^{2+}, also have a great influence on NO_3^- uptake.

In contrast to NH_4^+, under a low pH condition, absorption of NO_3^-–N will lead to the increase of the pH value in rhizospheres. In alkaline soils, the increase of rhizosphere pH may bring about unfavorable effects on some mineral nutrients (such as Fe) absorption and utilization (Alloush et al., 1990). However, our results show that using NO_3^-–N as the sole N fertilizer, the pH in rhizosphere was increased by 0.2–0.4 pH units in a calcareous soil. Although this was not beneficial to the NO_3^- uptake, wheat still had a good response to NO_3^-–N than to NH_4^+–N. It seems that the good effect of NO_3^-–N was due to its function and not due to its absorption in such a soil.

4.3.2. *Factors Affecting NO_3^- Uptake and Assimilation*

Supplying sufficient NO_3^-–N, NO_3^-–N contents in stems and roots can reach 100 mmol dm^{-3}, most of which gets stored in vacuoles. Cytoplasm is an important site for many physiological and biochemical reactions. Determination of the NO_3^-–N content in the cytoplasm depends on the methods or techniques used. Adoption of compartmental analysis of the NO_3^-–N content in the entire tissue shows that the contents vary greatly. When the environmental concentration of NO_3^-–N ranges from 0.01 to 1 mmol dm^{-3}, the determined values of barley roots shifted from 10 to 35 mmol dm^{-3}. Specific electrodes can be used to directly measure cell NO_3^-–N concentration, and the result is relatively stable (Siddiqi et al., 1991). It has been found that in maize and barley, when the environmental NO_3^-–N concentration varied between 0.1 and 10 mmol dm^{-3}, cell NO_3^-–N concentration stabilized at 3 and 4–5 mmol dm^{-3}, respectively (Miller and Smith, 1996).

4.3.2.1. Uptake and Assimilation of NO_3^-–N

The NO_3^-–N enters the cortex symplast from the epidermis and mainly proceeds in four ways by (1) getting reduced to NO_2^- by NR in the cytoplasm and further getting assimilated to amino acids; (2) moving to the apoplast; (3) getting stored in vacuoles; (4) entering the xylem (Crawford and Glass, 1998). When NO_3^-–N is transported to the leaves, it changes in diachyma (mesophyll) cells as it changes in roots. The concentration of NO_3^-–N is high in the xylem sap, reaching 5–40 mmol dm^{-3} while it is low in leaf blades, illustrating that diachyma cells are able to assimilate NO_3^-–N effectively.

4.3.2.2. Carbohydrate

The NO_3^- assimilation is linked to the availability of sugars to provide C skeletons. Some physiological studies show that sugar contents have some relations with NO_3^- uptake (Delhon et al., 1995). The uptake velocity of NO_3^- by soybean is higher in the photoperiod than in the dark period. In the case of removal of CO_2 in the atmosphere or implementation of ring cutting of the stem to stop sugar moving to the roots, the light stimulation to NO_3^- absorption is inhibited while the supply of sugar to the roots prevents the inhibition. Plants grown in lower optical density or in shorter duration of sunshine contain very high NO_3^- (Matt et al., 1998; Stitt and Krapp, 1999). It has been found that there is a close correlation between light intensity and NR in green leaves, and this may reflect fluctuations in carbohydrate concentration and in the corresponding supply of reducing equivalents and C skeletons (Anjana and Iqbal, 2007). The diurnal fluctuations in NRA may relate to the decrease in the foliar NO_3^- concentrations during the light period (Neely et al., 2010). Plants grown permanently under low-light conditions such as in greenhouses during winter may contain NO_3^- concentration several folds higher than those grown under high-light conditions such as in an open field during the summer. This is particularly evident in certain vegetables belonging to Brasisicaea or Chnepodiacea (Santamaria, 2006). For example, spinach (*Spinacia oleracea*) has a high preference for NO_3^- accumulation in the shoots and use NO_3^- accumulated in vacuoles for osmoregulation. Under low-light conditions, NO_3^- concentration in spinach leaves can reach 100 mM NO_3^-, corresponding to 6000 mg kg^{-1} fresh weight (Burns et al., 2011). All this shows that when the carbohydrate content is lower, the NO_3^-–N assimilation is more inhibited than its uptake, and the energy needed for absorption of NO_3^-–N is far lower than that for assimilation, and therefore, a very large pool of

NO_3^-–N may be formed in plants, and when the photosynthesis condition is improved, the NO_3^-–N will be rapidly used.

4.3.2.3. Effect of CO_2 Concentration on NO_3^- Assimilation

Carbon and nitrogen are two extremely essential and important nutrient elements for plant growth. The metabolism of N is closely related to C metabolism (Cramer et al., 1993; Farquhar et al., 1980), because both need CO_2 directly or indirectly as a basic raw material for synthesis of organic compounds, such as carbohydrates (Marschner, 1995; Mengel and Kirkby, 2001). The process for production of carbohydrates from inorganic substances, carbon dioxide, and water, known as photosynthesis, proceeds only with the absorption of light that supplies the necessary energy. The reduction of NO_3^-–N to NH_4^+–N by NR catalysis is the first step for its assimilation, since only when NO_3^-–N is transformed into NH_4^+, can the assimilation process be conducted. The assimilation of NH_4^+ requires a large amount of C skeletons for forming amino acid, and further for protein synthesis. Following the transformation of NO_3^-–N into NH_4^+–N during the process of N assimilation, C skeletons would be increasingly needed. For producing such C skeletons, fixation of CO_2 through photosynthesis is the basic step and is of great importance. The increase of CO_2 concentration exerts a great influence on plant photosynthesis. With the increase in photosynthesis, C skeletons certainly increase, and thus N metabolism, and particularly NO_3^- metabolism is affected. It has been reported that the net C fixation in roots was higher with NH_4^+–N compared to NO_3^-–N supply by up to threefold in rice and tomato (Ikeda et al., 1992) and fivefold in maize (Cramer and Lewis, 1993). It can be reasonably assumed that an increase in CO_2 concentration will increase plant photosynthesis, produce more C skeletons, and therefore promote NH_4^+–N assimilation. With the increase of NH_4^+–N assimilation, the reduction of NO_3^-–N in plants should be promoted and accelerated.

Studies on the relation of NO_3^-–N metabolism to carbon metabolism and the relation of CO_2 and NO_3^-–N have been noted for a long time. Le et al. (1991) applied NO_3^-–N to detached wheat leaves; the CO_2 assimilation was reduced by 21% and sucrose synthesis by 51%. This shows that a competition existed between them. In contrast, Robinson (1988) used spinach to conduct an experiment under saturation of light intensity and found that the NO_3^- metabolism did not affect CO_2 assimilation. Romero and Lara (1987) reported that when both light intensity and CO_2 concentration were saturated, application of NO_3^- had no obvious effect on the CO_2 assimilation

of cyanobacteria (cyanophyta cell). All this indicates that there is no competition between them, or even if there is such a relation, the competition is too small to play any significant role. Some experiments found that when CO_2 concentration was elevated, pine (*Pinus*) seedling increased its ability for the uptake of NO_3^-–N (Johnson et al., 2000; Stitt and Krapp, 1999), and N concentration in plants was increased (John et al., 2000), and so was the photosynthetic velocity and N use efficiency (Sun et al., 2002). These results show that there is a promotion relation between NO_3^-–N and CO_2. Based on the different results, some researchers consider that the relationship between CO_2 and NO_3^-–N is associated with light. Under saturation of light, NO_3^-–N metabolism did not influence CO_2 assimilation, while under weak light conditions, application of NO_3^-–N inhibited CO_2 assimilation, and under strong light conditions, NO_3^-–N metabolism promoted CO_2 assimilation (Angel and Delgado, 1991; Obinson, 1986; Tian et al., 2001). In such studies, attention has been paid to the effect of CO_2 elevation on N, P, and K absorption by plants (Larios et al., 2004; Li and Kang, 2002a, 2002b).

Due mainly to human activities, the earth's atmospheric CO_2 concentration has increased by 30% since preindustrial times (Petit et al., 1999), and was increased at a rate of 1.8 μmol mol^{-1} CO_2 per year in the past (Neftel et al., 1985). However, it increased by 2.0 μmol mol^{-1} per year during 2000–2009 (Pieter, 2011; SIO and NOAA, 2012), and it was approximately 392 μmol mol^{-1} at the beginning of 2011. The concentration with respect to the preindustrial concentration of 280 μmol mol^{-1} has increased roughly exponentially with a growth rate of 2.2% per year in the past decades (Pieter, 2011; SIO and NOAA, 2012). Carbon dioxide is essential to plant photosynthesis and other photoautotrophs, and is also a prominent greenhouse gas. Despite its relatively small overall concentration in the atmosphere, CO_2 is an important component of the earth's atmosphere because it absorbs and emits infrared radiation at wavelengths, thereby playing a role in the greenhouse effect, in addition to other factors such as water vapor. The present level is higher than that at any time during the last 800 thousand years and is likely higher than that in the past 20 million years (Amos, 2010; Canadell et al., 2007; Gerlach, 1991; Page et al., 2002; Petty, 2004). It is estimated that by the end of this century, the atmospheric CO_2 concentration may reach 650–700 μmol mol^{-1} (Ye and Chen, 1992). If so, great changes can occur in the globe. The increase in CO_2 in the atmosphere will affect various aspects of plant growth. The N metabolism is one of such aspects (Johnson et al., 2000). Research has shown that plant absorption of mineral N

from soil and/or fertilizers was accelerated with the increase of CO_2 concentration (Prior et al., 1998; Stitt and Krapp, 1999). The inhibitor of carbonic anhydrase can reduce NO_3^- uptake by roots with normal concentration of CO_2, while no such function is reported with the CO_2 enrichment (Prior et al., 1998). Some results show that when the CO_2 concentration in the surrounding atmosphere is doubled, the uptake rate and speed of NH_4^+ cations by roots of *Acer rubrum* are increased, whereas no such effect is observed on *Acer saccharum*, soybean (*Glycine max*), and sorghum (*Sorghum bicolour*). This shows that the effect is species dependent. van der Merwe and Cramer, 2000 reported that the enrichment of CO_2 around roots of tomato (*Lycopersicon esculentum* L. Miu. cv. F144) cultured in nutrient solution had increased an NO_3^- uptake by 30% whereas NH_4^+ uptake did not increase. Bassirirad et al. (1996) showed that when the CO_2 concentration was increased, the seedlings of *Loblolly pine* increased NO_3^-–N uptake, but NH_4^+–N uptake was not influenced. Although the results obtained by different investigations are not consistent, one thing is certain that the increase of CO_2 concentration affects N metabolism.

Men and Li (2005a, 2005b) transplanted winter wheat (*T. aestivum* L. cv. Xiaoyan 22) plants at heading to milking stages to Hoagland's cultured solution in which NO_3^-–N was the sole N source to investigate the effects of CO_2 concentration and light intensity on the absorption, reduction, and transfer of N metabolism of roots and shoots in relation to NO_3^-–N and NH_4^+–N concentrations and NRA in plants as well as N uptake from solution.

Results show that regardless of whether shading was adopted or not, increase of CO_2 concentration significantly decreased plant shoot NO_3^-–N concentration. Under natural light conditions without the addition of CO_2, the shoot NO_3^-–N concentration remained higher while with the addition of CO_2 it decreased and remained lower. Trends under shaded conditions were usually similar to those under the normal light condition (Fig. 5.3). With a reduction in NO_3^-–N concentration in plants, the NRA trended to decrease. The results were similar to those obtained by Larios et al. (2004) in sunflower study, and also agreed well with those of other studies (Johnson et al., 2000; Stitt and Krapp, 1999). Their results further showed that under natural light conditions, shoot NH_4^+–N concentration decreased from 77 $\mu g\,g^{-1}$ FW without the addition of CO_2 to 60 $\mu g\,g^{-1}$ FW with the addition of CO_2 while under the shaded condition, there was no significant difference; it was 55 and 56 $\mu g\,g^{-1}$ FW, respectively, for the two CO_2 treatments. In contrast, the total plant uptake N was increased for CO_2 addition treatment, and the increased total N agreed well with the decreased

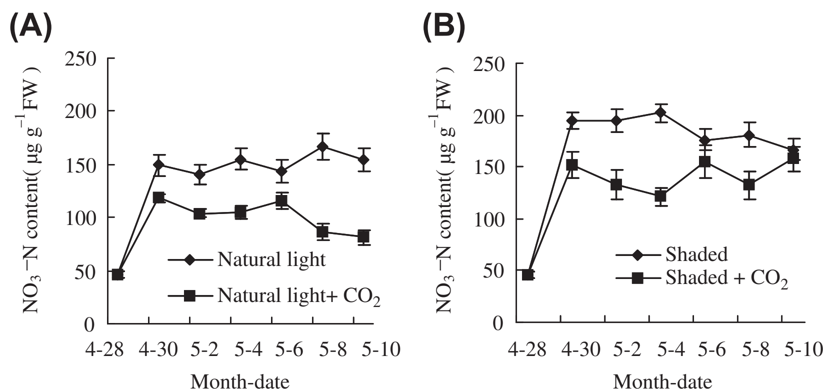


Figure 5.3 Effect of CO_2 concentration on NO_3^-–N concentrations of wheat in the presence of light (A) and in shaded (B) conditions. *Men and Li (2005a, 2005b)*.

amount of NO_3^-–N in the solution. Based on the fact that plants absorbed more NO_3^-–N from solution (Table 5.4) and had more total N in their tissue (Table 5.5) with the addition of CO_2, it was logical to conclude that the reduction of NO_3^-–N and NH_4^+–N in plants was not caused by either the decrease of N uptake or the decline of NH_4^+ formation, but by the increase of NO_3^-–N assimilation and formation of more organic N compounds, and the shaded condition reduced N assimilation of plants by decline of N uptake compared to the natural light condition. The findings directly support the conclusion that the increase of CO_2 concentration increased NO_3^-–N uptake, and also suggest that the increase of CO_2 be beneficial for NH_4^+ metabolism.

4.3.2.4. Transport of NO_3^-–N and Amino Acids

The demand of N by plants may be defined as the difference between the organic N amount transformed and that required for maintaining optimal growth of plants. The ions in root cells regulate the synthesis of transport proteins that transfer these ions, and thereby encourage the absorption of the ions. Since the transport form of N in the phloem is not NO_3^-, and thus changes of NO_3^- from roots cannot provide information of N needed by shoots. In general, the net NO_3^--absorption by plants is far lower than their absorbing ability, indicating that NO_3^-–N uptake occurs via a negative regulation signal. Such a regulation is completed mainly through the amino acid cycle of roots and shoots. For example, at the vegetative growth stage of soybean, the vegetative storage protein (VSP) increase is positively correlated with the NH_4NO_3

Table 5.4 Effect of Light and CO_2 Enrichment on NO_3^-–N Uptake of Winter Wheat From Solution

Treatment	Concentration of N in solution (mg N L^{-1})		Total N absorbed from solution (mg N pot^{-1})	Uptake of N per plant (mg N $plant^{-1}$)
	April 28	May 10		
Natural light	210	101	109 ± 2.5	24.3b*
Natural light + CO_2	210	87	123 ± 5.2	27.3a
Shaded	210	154	58 ± 3.3	12.9d
Shaded + CO_2	210	143	67 ± 2.6	14.8c

*Different letters following the numbers in the column indicate significance at the 5% level tested by Duncan's method under the protection of variance analysis.
Men and Li (2005a, 2005b).

Table 5.5 Effect of Light and CO_2 Enrichment on Plant N Uptake of Winter Wheat

Treatment	N content (g kg^{-1}) in wheat shoots	Shoot dry weight (g $plant^{-1}$)	N uptake (mg N $plant^{-1}$)	
			Total	Within 12 days
April 28 (before treatment)				
	40.3	1.85 ± 0.16	74.6	0.0
May 10 (treated with different CO_2 concentrations and light intensities for 12 days)				
Natural light	45.5	2.2 ± 0.04*	99.2	24.6b**
Natural light + CO_2	46.7	2.2 ± 0.02	104.7	30.1a
Shaded	39.8	2.1 ± 0.08	84.0	9.4d
Shaded + CO_2	43.1	2.1 ± 0.13	89.7	15.1c

*The standard deviation of the mean.
**Different letters following the numbers in the column indicate significance at the 5% level tested by Duncan's method under the protection of variance analysis.
Men and Li (2005a, 2005b).

use in media; at the podding stage, NO_3^- uptake is rapidly declined and so is the VSP content in leaf blades. It can be seen from these results that NO_3^- absorption is parallel to the synthesis and degradation of VSP (Staswick et al., 1991). An experiment by soaking cotyledon of seedlings into media that contain amino acids (Muller and Touraine, 1992) shows that Arg, Ala, Asn, and Gln strongly inhibits NO_3^- absorption, while Glu, Met, and Asp had weak inhibition and some other amino acids had no effect on NO_3^- uptake. These results are similar to those obtained by the direct supply of amino acids to roots, and illustrate that the cycles of amino acids or polypeptides in the phloem may control root uptake of NO_3^-.

4.3.2.5. pH

The change of cell pH mainly induced by organic acids is another factor controlling NO_3^- absorption. During the process of NO_3^- reduction, the alkaline ions formed cannot be completely and simply released (eliminated) to outside cells. For maintenance of pH balance in cells, plants must synthesize strong organic acids, mainly oxaloacetate. The leaf blade is the main place for NO_3^- reduction. Supplied with NO_3^-–N, the NO_3^- reduction produces far larger negative charges than does the carboxylic group, and the negative charges are mainly formed due to the transport of the carboxylic group. The carboxylic group is transported mainly as potassium malate from the phloem and decomposed in roots, and HCO_3^- is released from roots, while potassium ion (K^+) enters the new cycle accompanying the absorbed NO_3^- re-entrance to the xylem and is then transported to leaf blades. The transport velocity of potassium malate determines the NO_3^- uptake and reduction. Injection of malate to root surfaces or the phloem can change NO_3^- uptake velocity, and therefore, the stimulation or inhibition of the reductive potential of NO_3^- in leaf blades will influence the absorption of NO_3^- by roots (Thibaud and Grignon, 1981; Touraine et al., 1992).

It seems apparent that the hypothesis of amino acid regulation is in conflict with that of organic acid regulation. In fact, it is not because two substances are not simultaneously transported outward. Using soybean as an example, at the vegetative growth stage, NO_3^- reduction, synthesis of carbohydrates and amino acids are all high, but the majority of amino acids is used to synthesize chlorophyll, ribulose-1,5-bisphosphate carboxylase/oxygenase, and VSP, while carbohydrates are transported to roots. Therefore, when high concentrations of HCO_3^- exist, the amino acid concentration in the phloem is very low. At the podding stage, the protein in leaves is hydrolyzed, NO_3^- is reduced, and so is HCO_3^- that could be used. However, due to the redistribution of N, amino acids transported from leaves increase, and a large portion of transported amino acids goes to the pods. Due to very rapid cycle velocity of N in the xylem–phloem (Touraine et al., 1992), the phloem also contains abundant amino acids. As a result, the NO_3^- absorption velocity of the roots is decreased due to the reduction of carbohydrates and the increase of amino acids.

The uptake rate of the accompanying cation also affects the proportion of NO_3^- reduced in shoots. With K as the accompanying cation, translocation of both K and NO_3^- to shoots is rapid while correspondingly,

NO_3^- reduction is relatively low (Ruiz and Romero, 2002). In contrast, when Ca or Na is the accompanying cation, NO_3^- reduction in roots is increased significantly (Cramer et al., 1995).

4.3.3. Storage of NO_3^-–N in Vacuoles

NO_3^- is more readily distributed throughout the plant compared to NH_4^+ (Dechorgnat et al., 2011). In addition to reduction into NH_3 in plants and then being utilized, NO_3^- can be accumulated in such a form in the vacuole of roots, shoots, and storage organs from where it can be retrieved (Rossato et al., 2001) relative to the cytosol (Miller and Smith, 1992). From the physiological point of view, the principal cause for NO_3^- accumulation in vacuoles is NO_3^- uptake exceeding NO_3^- assimilation by plants. In other words, plants take up too much NO_3^-–N, and it is impossible for plants to rapidly reduce it into NH_3. The high uptake is related to the high supply from the media or the soil. No matter what the mechanism of absorption of NH_4^+ and NO_3^- is, the velocity of crop uptake of NH_4^+–N or NO_3^-–N is closely related to each concentration in media. Such a relationship can be described by Michaelis–Menten kinetics that was developed to describe enzyme reaction rates (Barber, 1984a; Ni, 1982; Yang and Sun, 1991a, 1991b). The other reason is a shortage of carbohydrate that will lead to the reduced NH_4^+ unable to be used (Du et al., 2007; Gruda, 2005; Li et al., 2009).

It has long been realized that in plants, there exist two pools, metabolic pool and storage pool, of NO_3^- in plant cells. The cytoplasm is characterized in metabolism, and therefore, it acts as the metabolic pool (Chen et al., 2002) and the vacuoles apparently represent the major repository for the storage pool of NO_3^- (Granstedt and Huffaker, 1982). Of the NO_3^-–N that enters plants, only part exists in the cytoplasm for metabolism, while part, maybe a large part, is stored in vacuoles. The NR exists in the cytoplasm, and the entered NO_3^-–N can be reduced into NH_3. Therefore, it is impossible for NO_3^-–N to exist in the cytoplasm for a long time, and the concentration of NO_3^-–N in the metabolic pool is low and stable without great changes. In general, this pool only occupies a small portion of the total NO_3^-–N in cells. Vacuoles are the normal components of the cell cytoplasm. In mature cells, vacuoles occupy 80% of the cell volume. The vacuole plays many roles in cell life activities, and one role is to store organic and inorganic substances, such as organic acids, amino acids, mineral salts, NH_4^+–N in addition to NO_3^-–N. Different from the metabolic pool, there is no NR existence in the storage pool. Therefore, the entered NO_3^-–N can exist in the pool for a relatively long time. It is clear that in higher plant cells, the

distributive regionalization (compartmentation) characteristic of NO_3^-–N and NR is the main cause for NO_3^-–N accumulation: NR exists in the cytoplasm (metabolic pool) with a small amount of NO_3^-–N, while no NR exists in vacuoles (storage pool) where there is a large amount of NO_3^-–N.

The accumulation of NO_3^-–N in vacuoles is a comprehensive result of various factors such as NO_3^- uptake, transport velocity, and reductive capacity (Solomonson and Barber, 1990). The proportion of NO_3^- in the vacuoles at a given time is affected by several factors, and the accumulation of NO_3^- in vacuoles is a function of the rate of NO_3^- uptake from soil solution and the rate of reduction (Aslam et al., 1979). The fact that more NO_3^- accumulates in darkness than in light (Aslam et al., 1979) shows that photosynthesis plays a significant role in NO_3^- reduction and therefore in its accumulation. As an important factor influencing photosynthesis, light also stimulates the release of NO_3^- from storage pools into metabolic pools (Aslam et al., 1976).

The amount of NO_3^- accumulated in vacuoles is also related to plant species. Some plant species such as members of the families Chenopodiaceae, Compositae, Amaranthaceae. Solanaceae, and Cruciferae tend to store large quantities of NO_3^-, and sometimes, the amount can be up to 5% of tissue dry weight (Schrader, 1978). In some plants, release of storage NO_3^- from vacuoles can supply enough N to sustain growth for several days (Novoa and Loomis, 1981). In species of salt bush (members of the Chenopodiaceae), the uptake of NO_3^- is particularly active during periods of rainfall, and NO_3^- that builds up in the plant at these times is slowly used during periods of drought (Pate, 1980). In crop plants, as a whole, it has been evident that 58–99% of NO_3^-–N accumulated in cells exist in vacuoles, and the amount of NO_3^-–N in plant tissues is also determined by the concentration of NO_3^-–N in vacuoles. Such a difference does not only exist in different species but in different cultivars of the same crop as well. Such characteristics of NO_3^- accumulation can be inherited to the new generation (H.B. Du et al., 2001; X.M. Du et al., 2001). Therefore, the different properties of plants in the uptake and assimilation of NO_3^-–N have a great influence on NO_3^-–N accumulation.

The metabolic pool and the storage pool are not independent kingdoms. Although different in functions, they keep either information exchange or material exchanges. When nutrients in the metabolic pool are deficient, the substance in the storage pool will enter the metabolic pool to participate in metabolism. For this reason, NO_3^- stored in the vacuole is a reservoir of N to be used when the external N supply is low (Richard-Molard et al.,

2008; Van der Leij et al., 1998), and vacuole NO_3^- is rapidly remobilized to sustain cytosolic NO_3^- concentration when plants are deprived of external NO_3^- or when NRA is high at high light intensities (Cookson et al., 2006). Accordingly, the rate of release of NO_3^- from the vacuole in leaf cells does not appear to be a rate-limiting step for the use of stored NO_3^-. As a result, the nutrient exchanges in the two pools are continuously conducting. For this reason, the storage of NO_3^- in vacuoles is of significance for plant growth without obvious toxicity and the stored NO_3^- in leaf vacuole can enter the cytoplasm and participate in metabolism when the initial NO_3^- in cytoplasm is used up. At last, the entire NO_3^- will be further assimilated into various organic N compounds, particularly amino acids. The amino acids formed in the root system can also be transported to the shoots and form protein in leaves, and those formed in leaves can be transferred to root system through sieve tubes. However, NRA peaks when the rate of leaf expansion is maximal and becomes very low in fully expanded leaves (Reed et al., 1980); this may limit use of stored NO_3^- in senescing leaves. In roots, NRA is high in expanding cells of the apical zones and decline rapidly toward the basal root zones (Di Laurenzio et al., 1996). In addition, this N reserve is, in most cases, very small compared to organic N in plants, and the store of NO_3^- in the vacuole is depleted within 12–48 h of NO_3^- starvation (Richard-Molard et al., 2008). NO_3^- is stored in vacuoles transiently, for example, during the night when NO_3^- is not metabolized by NR. This suggests that the storage of NO_3^- in vacuoles serves as an NO_3^- buffer for transport process rather than as N storage.

Why do plants luxuriously absorb NO_3^- and accumulate it in the vacuoles, but not absorb only the amount that can meet plants need? This is caused by plant demand and has important physiological significance. An excessive accumulation of NO_3^-–N occurs mainly in the vegetative growth stage, especially the seedling stage, not only in vegetables but also in other crops. Our research shows that the excessive accumulation of NO_3^-–N in the vegetative growing stage is a common property for all plants. With a sufficient supply of nutrients, at the vegetative stage, plants excessively absorb (exceeding plant reduction ability) NO_3^- and NO_3^-–N accumulated in wheat stems and leaves is as high as or even higher than some vegetables. The accumulated NO_3^-–N in plants can not only ensure current plant normal growth and development but can also ensure later stage need when N nutrient supply in soil is deficient. The more the NO_3^-–N accumulates, the better the plant growth and development at late stage. Therefore, the plant accumulation of NO_3^-–N at early stages is a "storing" measure and also

a phenomenon of adaptation to environments for guaranteeing the nutrient requirement of plants at later stages. With growth of crops, NO_3^-–N in plants becomes gradually reduced (H.B. Du et al., 2001; X.M. Du et al., 2001; Li et al., 2009). In addition, NO_3^- in vacuoles is also an important osmotic-regulating substance. When the synthesis of carbohydrates is declined and the organic substances in vacuole are decreased, NO_3^- can play a role in osmotic regulation instead of the organic substances, and such a regulation also needs less energy.

4.4. Assimilation of NH_4^+/NH_3

NH_4^+ or NH_3 is a central intermediate in plant N metabolism. Besides uptake by plant roots from the soil, NH_4^+ is constantly generated at high rates in plant tissues by processes such as NO_3^- reduction, photorespiration, lignin biosynthesis, senescence-induced N remobilization, and N_2 fixation in legumes (Joy, 1988). Irrespective of the source of NH_4^+ or the organ in which it is assimilated (roots, root nodules, and leaves), it is rapidly assimilated in plants.

4.4.1. Ammonia Assimilation

In addition to transporting to shoots with a transpiration stream through the xylem and storing in vacuoles of roots and shoots, NH_4^+ entering plant roots can be directly assimilated with only 296 light quanta being consumed for assimilation of 1 mol NH_4^+–N (Raven, 1985). NH_3 is assimilated by plants under the catalytic action of glutamine synthetase (GS) and GOGAT (also known as glutamate synthase) or in one word, the GS–GOGAT enzyme system. Existing in the cytoplasm and plastids of the green tissue (mainly leaves) or nongreen tissue (roots) of higher plants, assimilation of most NH_4^+ derived from NH_4^+ uptake, N_2 fixation, NO_3^- reduction, and photorespiration is mediated by the GS–GOGAT pathway in which the amino acid glutamate acts as the acceptor for NH_4^+ and forms the amide Gln. The GS–GOGAT pathway is complex and occurs in two steps: the first step requires ATP to add NH_3 to glutamate to form Gln. The second step transfers the NH_4^+ from Gln to α-ketoglutarate to form two glutamates. The net reaction is as follows:



$$\text{Glutamate} + \text{2-Oxoglutarate} + NH_4^+ \rightarrow \text{2-Glutamate} + H^+$$

Once NH_3 has been incorporated into glutamate, it can be transferred to other carbon skeletons by various transminases to form additional amino acids.

With multiple metabolic functions, the cytosolic GS exists in multiple enzyme forms located in the cytosol and in plastids (Bernard et al., 2008; Hirel and Lea, 2001) and mainly catalyzes glutamic acid (Glu) with NH_4^+ to form Gln functioning as a key enzyme for promotion, of assimilation, transport, and distribution of NH_3 throughout the plant. During leaf senescence after anthesis, the GS plays a key role in the assimilation and recycling of NH_4^+ generated from various catabolic processes (Masclaux-Daubresse et al., 2007) for grain development and filling of cereals when N is remobilized to the reproductive sinks (Martin et al., 2006). Several isoenzymes of the GS1 gene family are abundantly expressed in roots with different *K*m values (Ishiyama et al., 2004a, 2004b) under N deficiency or under high external NH_4^+ supply. In chloroplasts, light-stimulated NO_3^- reduction and the enhanced NH_4^+ assimilation are coordinated through the import of 2-oxoglutarate from the stroma and export of glutamate from the stroma of chloroplasts into the cytoplasm, thus preventing high NH_4^+ concentrations. High pH and high concentrations of Mg and ATP increased in the chloroplast stroma on illumination can activate chloroplast GS. In cytosol and chloroplasts, GS is also subject to posttranslational regulation by phosphorylation and subsequent interactions with proteins (Finnemann and Schjoerring, 2000; Lima et al., 2006).

The GOGAT, the other enzyme involved in NH_4^+ assimilation, catalyzes the transfer of the amide group ($-NH_2$) from Gln to 2-oxoglutarate that is a product of the TCA cycle. The conversion of Gln to glutamate takes place in plastids that have two isoforms of GOGAT with one from reduced ferredoxin (from photosystem 1) for accepting electrons and the other from NADPH from respiration. The ferredoxin-linked GOGAT isoform dominates in leaves, particularly in the chloroplasts of phloem companion cells in leaf veins (Masclaux-Daubresse et al., 2007).

For many years, scientists assumed that there existed another way to be involved in NH_3 assimilation. This was the glutamate dehydrolysis: under the catalysis of GDH, NH_3 was synthesized with α-ketoglutaric acid to form α-amino acid and further form Glu (Lightfoot, 2009; Lightfoot et al., 1988, 2007; Mungur et al., 2005; Wootton, 1983). However, it is now evident that GDH is mainly involved in the liberation of NH_4^+ during senescence via catalyzing the oxidative deamination of glutamate, thereby providing carbon skeletons for respiration and oxidative phosphorylation. For this reason, the GDH enzyme, in conjunction with NADH-GOGAT, contributes to the control of leaf glutamate homeostasis, an amino acid that plays a central role in signaling at the interface of the C and N assimilation pathways (Labboun et al., 2009).

4.4.2. Low-Molecular-Weight Organic N Compounds

The glutamate and Gln formed by inorganic N assimilation can rapidly be used for the synthesis of other amides, amino acids, ureide, amines, and peptides. In higher plants, this process is nonreversible. All the compounds synthesized can be regarded as low-molecular-weight organic N compounds (LMWONCs) in contrast to proteins, nucleic acids, and other N-containing compounds that can be regarded as high-molecular-weight organic N compounds (HMWONCs). In higher plants, the LMWONC does not only act as intermediates between the assimilation of inorganic N and the synthesis of the HMWONC but also as the important compounds for transfer of N from source organs to sink tissues and to build up reserves during periods of high N availability (Hawkesford et al., 2011).

Glutamate, Gln, aspartate, and asparagine occupy a certain position in plants (Hawkesford et al., 2011). Indeed, Gln and glutamate are the major entry points of NH_3 into organic compounds, and the amino groups in glutamate and aspartate and the amide group in glutamine are the N source for most plant N compounds, including other amino acids (Morot-Gaudry et al., 2001). Aspartate is a metabolically reactive amino acid that serves as a donor in numerous aminotransferase reactions, while asparagine is relatively inert and serves primarily as an N storage compound (Hawkesford et al., 2011).

NH_4^+ assimilation in roots to synthesize amino acids has a large requirement for carbon skeletons provided by the TCA cycle, and the intermediates removed have to be replenished by increased activity of PEP carboxylase. Compared to the NO_3^- supply, the net carbon fixation in roots with NH_4^+ is several-fold higher (Viktor and Cramer, 2005). To minimize the carbon costs for root-to-shoot transport, the bulk of the N assimilated in roots is transported in the form of N-rich compounds with N/C ratios >0.4 (Hawkesford et al., 2011). It has been found in the xylem exudates of the roots that some amides and amino acids are produced as such N-rich compounds: the amides Gln (2N/5C) and asparagine (2N/4C) (Pate, 1975); the amino acid arginine (4N/6C); and the ureides allantoin and allantoic acid (4N/4C). To save carbon skeleton consumption, such compounds are ideal for transportation (Streeter, 1979) and therefore in phloem transport to developing fruits, the non-photosynthetic sinks, amino acids with an N/C ratio of >0.4 are the predominant N forms. During these processes, protons are released to supplement electric charges (Mengel et al., 1976).

Of the LMWONC, those used predominantly for long-distance transport or for storage in individual cells differ among plant families. Glutamine and asparagine are dominating transport amides in *Graminae*, while asparagine in legume species such as clover (*Trifolium*), Lucerne (*Medicago sativa* Linn), pea (*P. sativum* L), and lupin (*Lupinus* L), which have indeterminate nodules. In other legume species such as soybean [*G. max* (L.) Merr.], cowpea [*Vigna unguiculata* (L.) Walp.], and common bean (*Phaseolus vulgaris*,) characterized by determinate nodules, the majority of the fixed N transported in the xylem of modulated roots is incorporated into the ureides allantoin and allantoic acid (Pélissier et al., 2004). The concentrations of these transport amino acids vary and are modulated by factors such as light and N availability. Glutamine is mainly synthesized in the presence of light, while asparagine is preferentially synthesized in the dark (Hawkesford et al., 2011).

Amides and polyamides belong to an important class of LMWOC, their biosynthesis being mediated by decarboxylation of amino acids such as serine that forms the basis for synthesis of ethanolamine contained in the lipid fraction of biomembranes. Arginine is the main precursor for polyamines, the important secondary messengers (Kusamo et al., 2008). Putrescine is usually the dominating polyamine in plants and may constitute up to 1.2% of plant dry matter. The polyamine concentration is particularly high in meristematic tissues of plants supplied with high concentration of NH_4^+ (Gerendas and Sattelmacher, 1990) and under K deficiency (Hawkesford et al., 2011).

As another LMWONC, betaine (glucine betaine) is involved in osmoregulation and is important for the adaptation of plants to drought or salinity, acting as compatible solute to counteract the osmotic perturbation caused by high vacuole concentrations of inorganic ions such as Cl, and Na that would inhibit cytoplasmic metabolism (Ashrf and Foollad, 2007; Dennis et al., 1989; Hanson and Wyse, 1982; Ishitani et al., 1995; Maqsood et al., 2006; Missihoun et al., 2011; Murata et al., 1992; Phodes et al., 1989; Sakamoto and Murata, 2000; Sakamoto and Murata, 2002; Sithtisarn et al., 2009; Yang et al., 2008; Zhang and Li, 2005, 2007a, 2007b; Zhang et al., 2007c, 2009, 2011a, 2011b). Under salt or drought stress, its synthesis and accumulation particularly in the cytoplasm are strongly enhanced (Hawkesford et al., 2011).

It is known that there are up to 200 different amino acids in plants, but only about 20 of them are required for protein synthesis. Not much is known about the role of large number of nonproteinogenic amino acids

in plants. However, at least some of them are important for plant nutrition (Hawkesford et al., 2011). For example, nicotianamine has an effect on iron hemostasis and on phloem transport of Fe, Zn, and Mn (Ishimaru et al., 2010; Suzuki et al., 2008) as well as on the acquisition of Fe in graminaceous plant species (Suzuki et al., 2006).

4.4.3. High-Molecular-Weight Organic N Compounds

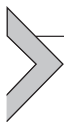
There are three ways for NH_4^+, no matter whether it is absorbed or is generated from other sources, to be used when it exists in plant roots: directly transporting in such a form to shoots with the transpiration stream through the xylem although the amount may be small; getting stored in vacuoles of roots, stems, and leaves to avoid its toxicity when there is a shortage of carbon skeletons; and getting assimilated in roots to form LMWONCs that may be transformed into HMWONCs in roots for in situ use, or it may enter vacuoles or may be transported to shoots and transformed there into HMWONCs for participating in plant metabolism. It has been proven that, of the LMWONCs, amino acids and amides such as Gln formed in roots can be transported to leaves and stems of shoots via bundles and then further form other complicated compounds such as proteins for plant use. For these reasons, plants often accumulate a little amount of NH_4^+. A ^{13}N-labeled experiment shows that when NH_4^+ enters roots, 41% and 20% ^{13}N remain in the cytoplasm and vacuoles, respectively, while 20% are moved out of the cells by efflux and 19% is assimilated. Martin (1971) postulates that NH_4^+ directly absorbed by roots is assimilated mainly in roots; a part may get transferred accompanied by malic acid to shoots to be assimilated. Therefore, the major forms for N transportation in the fibrovascular (bundle) system are amino acids, amides, and amines and their transferred amounts depend on N-absorptive sources and relate to root metabolism.

The LMWONCs, either formed in root cells or in other organs, will be further synthesized into HMWONCs such as proteins and nucleic acids (Allen et al., 1988). Such synthetic processes are extremely important for plant physiology since only through such syntheses could the plant structural materials be formed and the plant life be cycled. At the same time of synthesis, the enzymatic hydrolysis of high-molecular N compounds is carried out and participates in various physiological and chemical processes (He and Meng, 1987; Marschner, 1986; Mengel and Kirkby, 1987). The assimilation and hydrolysis processes of N include also pathways of N intertransformations of different major types of N components, and

these processes are affected by plant nutritional status, especially the soil N supplying capacity.

Increase in N supply will increase the different types of N components, but their magnitude is different. A large amount of N fertilizer addition will result in a great increase of the free, soluble amino-group compounds such as free amino acids, amides, and amines, while protein increase is limited (Haynes, 1986; Mengel and Kirkby, 1987) or unstable (Li, 1993). No matter whether NH_4^+–N or NO_3^-–N is applied, the N components are not significantly affected (Mengel and Helal, 1970), nor are the N accumulated amounts (Reddy and Reddy, 1993).

Since no reduction is needed as that in NO_3^-–N assimilation, the process of NH_3 assimilation reduces the consumption of photosynthetic products and ATP. However, compared to NO_3^-–N, the assimilation of NH_4^+ in roots consumes large amounts of carbon skeletons. This can be seen in the increase of PEP carboxylase activity (Arnozis et al., 1988) and oxygen consumption velocity of per unit weight root (Matsumoto and Tamura, 1981) and in the decrease of sugar contents in root systems. When the temperature and NH_4^+ concentration are higher, the plants supplied with NH_4^+–N grow poorly (Kafkafi, 1990). In roots, the assimilation of 1 mol NH_4^+–N will produce 1 mol of protons that must be exuded from the plant body. If the pH is low in the environment, the net release of proton will be inhibited, and the cell pH will decline (Gerendás et al., 1990). The low pH benefits polyamine synthesis (Smith and Sinclair, 1967) but is not beneficial to the root growth.



5. PREFERENCE OF PLANTS TO NO_3^-–N AND NH_4^+–N

There are many ways for improvement of N nutrition and N use efficiency, and one important way may pertain to N forms. Although in the environment, N exists in various forms, plants, as a whole, mainly take up inorganic N, and NH_4^+–N and NO_3^-–N are the major forms. The ions of NH_4^+–N and NO_3–N uptake by plants occupy >70% of the total amounts of cation and anion taken up by plants (Caicedo, 2000). Both NH_4^+ and NO_3^- can be very well absorbed by plants, and their assimilative processes are almost the same except that NO_3^-–N must be reduced to NH_3 before entering the synthetic and metabolic processes. For this reason, during a long period of time, agricultural workers have considered that both have an equivalent nutrient function and the same effect on crop yield on the basis of the same amounts of N. However, some results show that this is not

always true. NO_3^-–N and NH_4^+–N may exhibit different effects on plant nutrition, growth, and crop production for different plant species (Errebhi and Wilcox, 1990; Warncke and Barber, 1974a, 1974b), and therefore, different plant species may have certain or different responses or preferences to the two N sources or forms (Cao and Tibbitts, 1993, 1994; Gentry and Below, 1993; Ismunadji and Dijkshoorn, 1971; Kirkby, 1967).

5.1. Types of the Preference of Crop Plants to NH_4^+ and NO_3^-

Plant preferences to NH_4^+–N or NO_3^-–N have been evaluated mainly on biomass production or on the N uptake amount by the application of NH_4^+–N or NO_3^-–N alone from early studies. Based on current results, crop plants may be classified into four types. It should be noted that the crop plant preference to NH_4^+–N or NO_3^-–N is the demand nature of plant species in particular, and in most cases, it is the result of plant demand and environmental combination, especially the medium pH. Although a large number of research studies on plant preference to the two N forms have been conducted for a long time, using water culture, pot experiment, and field trials, up to now, many matters have not been solved and different results and viewpoints still exist.

5.1.1. Preference of Crop Plants to NH_4^+–N

From the plant physiological view, assimilation of NH_4^+–N consumes much less energy than NO_3^-–N does, and therefore, it seems more economic to use NH_4^+. However, in most cases, NH_4^+–N for plant growth is less effective than NO_3^-–N (Cox and Reisenauer, 1973; Guo et al., 2002). The preference for NH_4^+ relative to NO_3^- increases strongly with decreasing temperatures, and at <5 °C uptake of NH_4^+ can still proceed while that of NO_3^- ceases (Macduff and Jackson, 1991). This may reflect the greater costs of metabolic energy associated with absorption and assimilation of NO_3^- compared to NH_4^+. However, sole NH_4^+–N nutrition may cause plant toxicity, rhizosphere acidification, cation uptake reduction, and organic acid synthesis decline and leaf expansion objections or hindrance (Magalhaes and Huber, 1991; Raab and Terry, 1994). As a result, only some plants, but not all plants, prefer NH_4^+–N.

Plant species differ in their tolerance to NH_4^+ (Britto and Kronzucker, 2002). Among crop plants, barley is NH_4^+ sensitive, while rice is NH_4^+ tolerant. Generally, plants adapted to soils that are acid (calcifuge species) or have a low redox potential (e.g. wetlands) have a preference for NH_4^+ (Lee, 1999). In acid soils, nitrification is often strongly inhibited (Ellenberg, 1977;

Havill et al., 1974; Osborne and Whittington, 1981). This has led to such results that NH_4^+–N is larger than NO_3^-–N and NH_4^+ rather than NO_3^- becomes the dominantly available N and therefore the major form for plant uptake. Adapted to such conditions, plants have a distinct preference for NH_4^+–N rather than for NO_3^-–N (Lee, 1999). Such plants may be deficient in their capacity to reduce large amounts of NO_3^-–N. Several workers have shown that many types of crop plants grown in acid soils prefer NH_4^+ –N, including some tree species, some grassland species and some crops (Haynes, 1986; Haynes and Goh, 1978; Townsend, 1966). Typical examples of forest tree groups most studied are some calcifuge or calciphobe (acid-loving) plants such as *Pinus massoniana*, *Dicranopteris dichotoma*, and *Lycopodium clavatum*. These plants mainly belong to the family Ericaceae, including species of the genera Azalea, Rhododendron, Erica, and Vaccinium (Havill et al., 1974; Routley, 1972; Townsend, 1966). Originated from acidic soil environments, these plants are characterized by growing only in calcium-deficit acid soils and generally much preferring, at least tolerating an N supply predominantly in the form of NH_4^+(Gigon and Rorison, 1972; Havill et al., 1974; Ingestad, 1970, 1971, 1973, 1976, 1979; Ingestad and Lund, 1979; Krajina et al., 1973). However, their preference to NH_4^+–N varies depending on the conditions where the plants live. Krajina et al. (1973), for example, found that of four tree species studied, two preferred NO_3^-–N, one preferred either NH_4^+ or NO_3^-, or a composition of NH_4^+ plus NO_3^-, and one had a preference for NH_4^+. In nature, those preferring NO_3^-–N grow in soils where nitrification occurs, while those preferring NH_4^+–N or either of the two N forms grow in soils where nitrification does not occur.

Very similar results have been observed for grassland plants (Gigon and Rorison, 1972; Wiltshire, 1973). As typical examples of grassland species, Barber, (1979a) (1979b) found that tall fescue (*Festuca* spp.) and reed canary (*Phalaris arundinacea*) grasses are NH_4^+–N preferring plant species and when NH_4^+–N and NO_3^-–N coexisted in equal amounts, NH_4^+–N uptake amount was 1.3- to 2.2-fold of NO_3^-–N. Some plants that adapt to low redox (oxidation–reduction) potential prefer NH_4^+–N as well, and rice is a typical representative (Ismunadji and Dijkshoorn, 1971).

The carbohydrate storage in plants affects crop responses to the absorption of NH_4^+–N and NO_3^-–N: crops containing high carbohydrates such as sweet potato (*Ipomoea batatas*), potato (*S. tuberosum* L.), and tea trees (*Camellia sinensis*) prefer NH_4^+–N, and application of NH_4^+–N can obtain better results. For an example, potato grows better in an NH_4^+–N: NO_3^-–N ratio of 3:1 (Bloom et al., 1993), and due to adaptation to low pH, supply

of NH_4^+–N promotes potato tuber growth even if the pH in the media is reduced (Gerendás et al., 1990). In contrast, plants containing low carbohydrates such as sugar beet (*Beta vulgaris*), tobacco, wheat, maize, sunflower (*Helianthus anuus* L), and hemp (*Cannabis sativa*) prefer NO_3^-–N (Beevers, 1976; Cheng, 1990; Fried et al., 1965; Ganmore-Neumann and Kafkafi, 1983; Kirkby, 1968; Loo, 1931; Michael et al., 1970; Prianishnikov, 1945; Sun, 1987). Prianishnikov (1945) claimed that shortage of sugar contents first inhibited the synthesis of amides, and then inhibited the reduction of NO_3^-–N. Plants such as garden sorrel (*Rumex acetosa*) or sharp dock, prefer NH_4^+–N since it can form NH_4^+ salts of organic acids to eliminate the toxicity of NH_3, and therefore is not toxic even if a large amount of NH_4^+–N has been taken up. It is believed that in acidic soils or waterlogged soils, the nitrification was weak, and therefore, NH_4^+–N was superior to NO_3^-–N.

Many results indicate that for rice, the application of NH_4^+–N alone or the application of NH_4^+–N at early stages whereas application of NO_3^-–N at a late stage could give the best results (Wen, 1993; Yang and Sun, 1990). Yang and Sun (1986) reported that the uptake velocity of NH_4^+–N was higher than that of NO_3^-–N for rice seedlings of a 4-week age, but the root affinity to the two N sources was not different. Yang and Sun (1991b) also indicated that during the reproductive growing stage, the uptake velocity of NH_4^+–N by rice was still higher, but the root affinity to NO_3^-–N was larger than that to NH_4^+–N. Using an isotope ^{15}N experiment, Fried et al. (1965) observed that when NH_4^+–N and NO_3^-–N equally coexisted in culture media or solutions, 2-week-aged rice seedlings took up more NH_4^+–N than NO_3^-–N, and the root absorptive velocity for NH_4^+–N was 5- to 20-fold (depending on the pH) higher than for NO_3^-–N. F. J. Stevenson (1982), F. R. Stevenson, (1982) believed that most of the N uptake by plants from the soils was NO_3^-–N, and most of the plants also like to take up NH_4^+–N. At early growing stages, plants generally took advantage of NH_4^+–N, while at late stages, NO_3^-–N was taken up in priority. No consistent result has been obtained for maize response to NH_4^+–N and NO_3^-–N. Coic et al. (1963) reported that maize took up NO_3^-–N as high as 90% of the total N absorbed, whereas others showed that maize yield was higher for the use of NH_4^+–N than for the use of NO_3^-–N. Warncke and Barber (1973) reported that compared to NO_3^-–N, application of NH_4^+–N to maize produced higher plant biomass. Ikeda and Osawa (1983) found that maize and strawberry were better grown with a supply of NH_4^+–N. Pot and field experiments from Li and Wang (1993) showed that the effect of NH_4^+N and NO_3^-–N on maize depended on

N amounts applied: at low rate, NO_3^-–N was better than NH_4^+–N, while at high rates, NH_4^+–N was the case. As a whole, NH_4^+–N was superior to NO_3^-–N. Riga et al. (1980) conducted experiments on a loess soil for 3 years and found the total cumulative recovery rate of ^{15}N% of maize for NH_4^+–N being larger than for NO_3^-–N, revealing that NH_4^+–N had a higher recovery rate and residual effect.

Soybean has nodules that can fix N from the atmosphere. However, at the early stage before nodule formation, soybean has no function for the fixation of N and at the vigorous growing stages, the fixed amount may not be enough to meet its demand. Therefore, application of a suitable amount of N is necessary (He et al., 1999). Like other crops, soybean also absorbs NH_4^+–N and NO_3^-–N as the main N source. Both NO_3^-–N and NH_4^+–N benefit soybean growth in fields, but different ratios have different effects on soybean growth. A water culture experiment from Vessey et al. (1990) showed that the absorbed ratio of NH_4^+–N to NO_3^-–N of soybean depended on the solution pH: at pH 6.0, the absorbed ratio was 2:1. However, Song et al. (1995) gave different results. Using a water culture experiment, they studied the effect of NO_3^-–N and NH_4^+–N, and their combinative ratio on soybean seedling growth and N accumulation. Results show that the combinative use of the two N forms was superior for soybean seedling growth to either of NH_4^+–N and NO_3^-–N alone. Using NH_4^+–N as the sole N source, plants showed damage symptoms: leaves in low positions were yellowed, and this color was gradually spread to the entire plant. The toxicity was affected by pH. At 5.5, serious toxicity occurred, whereas at pH 7.5, the toxicity was significantly alleviated, and the seedling growth was much better than when grown at pH 5.5. The use of NO_3^-–N alone or the mixture of the two N forms had made seedling growth less influenced by pH. Whether application of NH_4^+–N gives a higher growth rate and yield to some crops mainly depends on root temperature (Clarkson and Warner, 1979) and factors affecting carbohydrate supply to roots such as light intensity (Lavoie et al., 1992).

5.1.2. Preference of Crop Plants to NO_3^-–N

NH_4^+–N either from mineralization of organic matter or from ammonium and urea (amide N) fertilizers applied to the soil is able to be rapidly converted into NO_3^-–N through nitrification (Santamaria et al., 1998; Zhu and Wen, 1992) in most arable soils suitable or unsuitable for cultivation of plants, especially in dryland areas, calcareous soils, fertile soils of high pH lands, and good aerated soils, and therefore, NO_3^-–N becomes the major

and dominant mineral N form available to plants and is always taken up by plants in larger amounts than NH_4^+–N. It is estimated that these soils contain 6.0 mmol dm^{-3} of NO_3^-–N as an average, while only –0.77 mmol dm^{-3} of NH_4^+–N (Wolt, 1994). For this reason, NO_3^-–N is generally considered to be preferred by a number of higher plants (Nelson and Selby, 1974), and for the majority of plants, NO_3^-–N nutrition is superior to NH_4^+–N nutrition (Heberer and Below, 1989). Plants adapted to calcareous, high pH soils (calcicole species) use NO_3^- preferentially (Hawkesford et al., 2011). Tomato is generally regarded as a typical plant preferring NO_3^-–N (Zhao et al., 1998), and the NH_4^+–N nutrition declines its growth (Magalhaes and Huber, 1991). NH_4^+–N also decreases maize seedling growth (Magalhaes and Huber, 1991) and the biomass of potato, pea, and cucumber (*Cucumis sativus*) (Cao and Tibbitts, 1993; Raab and Terry, 1994). Due to the direct effect of nutrients on plant growth and development, application of different N forms results in great differences of N contents in different plant issues (Engels and Marschner, 1993). A type of plant called calcicole that thrives in soils rich in lime prefers NO_3^-–N (Haynes and Goh, 1978; Kirkby, 1967). The majority of dryland crops such as wheat, maize, cotton (*G. hirsutum* L), sunflower, hemp, sugar beet, spinach, Chinese radish or radish (*Raphanus sativus*), tobacco, proso (*Panicum miliacem* L.), millet [*Setaria italica* (L.) Beauv], sorghum (Coic et al., 1962, 1963; Cheng, 1990; He and Meng, 1987), and many vegetables, prefer NO_3^-–N, and the application of NO_3^-–N at the same N amount as NH_4^+–N has often given a higher yield; in strongly calcareous soil, NO_3^-–N effect on yield increase is more striking (Anderson et al., 1991; Li and Wang, 1993; Smiciklas and Below, 1992) while NH_4^+–N used as the sole N source easily induces toxicity and inhibit plant growth (Britto and Kronzucker, 2002; Britto et al., 2001). Hewitt and Smith (1975) proved that when supplied with NO_3^-–N, the growth of most plants was better. Using solution culture, Schjorring (1986) found that buckwheat, barley, and rape (*Brassica* species) grown for 23–34 days had a higher velocity to NO_3^-–N than to NH_4^+–N no matter whether P existed in solution or not. Also, on using top dressing or seed fertilizers to cereals, NO_3^-–N was found to be better than NH_4^+–N. With an increase of the N concentration in the environment, the superiority or the favorability of NO_3^-–N increases and the inhibition effect of NH_4^+–N to plants becomes clearer.

Wheat and maize are two major field crops in the drylands of China, and their response to N forms has received attention. Li and Wang (2003) conducted both pot and field experiments to study wheat response to NO_3^-–N and NH_4^+–N. Their results definitely show that wheat preferred NO_3^-–N

rather than NH_4^+–N, and application of NO_3^-–N as the sole N source or a combination of higher NO_3^-–N with lower NH_4^+–N such as a 2:1 ratio of NO_3^-–N to NH_4^+–N showed the best results in increasing crop yield and improvement of crop growth, while with NH_4^+–N as the sole N source, wheat yield was the lowest. The same trend was found in maize when the N rate was low (Li and Wang, 1993).

Why does NO_3^-–N commonly increase yields of wheat and maize more than NH_4^+–N on the drylands? Our results show that this is related to NO_3^-–N accumulation in plants. A large amount of NO_3^-–N accumulates in dryland areas (Santamaria et al., 1998; Zhu and Wen, 1992) and the external N-supplying status directly affects NO_3^-–N contents in plants. With the increase in NO_3^-–N in the soil, NO_3^-–N accumulation in plants is correspondingly increased (Ai et al., 2000a, 2000b; Du et al., 2007; Gruda, 2005; Wang and Li, 1996). When an NO_3^-–N fertilizer was applied to the soil, or when the soil accumulated a large amount of NO_3^-–N, wheat or maize could take up much more NO_3^-–N than normal and accumulate a large amount of NO_3^-–N in shoots. The increase of NO_3^- uptake and the excessive accumulation of NO_3^- in vegetative organs are common features for plants and occur at vegetative growth stages. This is true not only for vegetables but also for other crops. The difference between vegetables and cereal crops is that for vegetables, especially for leafy vegetables, the leaves and stems that accumulate large amounts of NO_3^- are used for food, while for the cereal crops, seeds that are used for food contain a small amount or no NO_3^- at all (Li et al., 2009). The high NO_3^- accumulation in cereal crop plants has several beneficial effects: (1) It promotes the transformation of absolute amount of NO_3^-–N into NH_4^+–N, and hence increases NH_4^+–N concentration. With an NH_4^+–N increase, amino acids, amides, and proteins are correspondingly increased (Li and Wang, 2003). Amide N is a storage form of N, and its high level favors N supply at a critical time when plants urgently need N (Prianishnikov, 1945); (2) ensures vigorous growth of plants. During the vegetative growth period, plants with high NO_3^- accumulation grow vigorously, and their leaves are green to dark green in color during the entire growing period. At later growing stages, plants still grow well, and the leaves remain green even if N supply from the soil becomes deficient, while those with a small amount of accumulated NO_3^- may grow poorly and leaves may become yellow. With continuous growth, the accumulated NO_3^-–N becomes significantly decreased, and at the flowering stage, plants with high NO_3^-–N accumulation still contain some NO_3^-, but those with low NO_3^- accumulation have almost no NO_3^- remaining.

As a result, the duration of plant growth period is elongated for plants with a higher NO_3^- accumulation. All this creates a favorable condition for plants to have abundant growth (Li and Wang, 2003; Li et al., 2009); (3) as the last result, high NO_3^- contents in soil promote plant uptake of more NO_3^-–N, and due to the beneficial effects of NO_3^-–N, plants have a higher dry matter and high yield.

These results have shown that under sufficient N-supplying conditions, plants can have excessive uptake of NO_3^- for use at later stages when the supply of NO_3^- from the medium has declined. Seen from numerous experimental results, we can conclude that the accumulation of NO_3^-–N in plants is a measure of N storage for adaptation to the detrimental environment or conditions plants may encounter. When a large amount of NO_3^- is accumulated, the plants can still grow vigorously even if the N supply from the soil is not sufficient at the later stage. The higher the accumulated NO_3^- is, the better the plant growth will be. From current research results, NO_3^-–N nutrition seems to offer more safety for maintaining plant growth than NH_4^+–N does. The accumulation of NO_3^- has no harmful effect on plant growth compared to that of NH_4^+–N, and this may be another reason why NO_3^- is a suitable form for plant storage.

Recently, we have conducted a number of field experiments to study the effect of NO_3^-–N and NH_4^+–N on wheat production. Through many field experiments, we have realized that for getting the correct answer from field results, avoidance of NO_3^- leaching by rainfall is substantially important. NO_3^- leaching by rainfall occurring after NO_3^-–N application will reduce NO_3^-–N availability to wheat, and result in different N amounts remaining in the soil profile available to plant roots. This will bring about an incomparable condition for evaluating the effect of N forms and lead to experimental failure. For this reason, our experiments were all carried out under conditions without NO_3^- leaching. The results show that NO_3^-–N was the major residual mineral N left in the soil profile, and NH_4^+–N was only one-fifth to one-eighth of NO_3^-–N. Wheat could absorb the residua N in the 100-cm layer, beyond which the residual N would be unavailable to wheat It is the NO_3^-–N amount accumulated in the 100-cm layer that does not only affect the N fertilizer result but also the N form result. In soils containing >150 kg NO_3^-–N per ha, N fertilization had poor and even no effect on wheat yield, and NO_3^-–N did not show better or only slightly better results on wheat yield compared to NH_4^+–N. By contrast, in soils containing <60 kg NO_3^-–N, addition of NO_3^-–N fertilizer showed a much better effect than that of NH_4^+–N did in the case where wheat responded

Table 5.6 Responses of Wheat Sown at Different Times to Ammonium N and Nitrate N

Sowing time (date/month)	Items determined	Yield (kg ha^{-1})		
		Control	Nitrate N	Ammonium N
Experimental location—Yongshou County, site 1				
23/9, 2010	Fresh biomass	17,666	21,122	19,933
	Dry biomass	12,720	15,419	14,452
	Seed yield	7200	8861	8222
16/10, 2010	Fresh biomass	8089	8947	8025
	Dry biomass	7118	8141	6861
	Seed yield	4335	4703	4332
Experimental location—Yongshou County, site 2				
23/9, 2010	Fresh biomass	13,316	14,445	13,240
	Dry biomass	11,851	13,217	11,916
	Seed yield	6678	7173	6564
16/10, 2010	Fresh biomass	7135	7789	7217
	Dry biomass	6421	7010	6560
	Seed yield	3728	4027	3773

Li et al. (2011) (Unpublished data).

to N fertilizers. In the latter case, application of NH_4^+–N (100 kg N per ha as designed for experiments) made the NH_4^+–N to be the dominant form in the soil (twofold larger than NO_3^-–N). At low temperatures that often occurred after wheat was sown, the NH_4^+–N applied would remain in the soil in such a form for a long time; this might be a stress to wheat. The addition of 100 kg NO_3^-–N per ha to soil, in contrast, further intensified NO_3^-–N nutrition. In this case, wheat seedlings contained higher N, wheat growth was more vigorous, and wheat had more tillers than on addition of NH_4^+–N. As a result, NO_3^-–N increased the wheat yield much higher than NH_4^+–N did. A strong evidence to demonstrate the different effects of the two N forms is obtained from wheat-sowing time experiments. Our other experiments showed that NO_3^-–N had a significant effect on the increase of wheat yield, whether wheat was sown either early or late , while the effect of NH_4^+–N on wheat yield increase was dependent on the sowing time: when sown early, NH_4^+–N significantly increased wheat yield although its effect was not as good as that of NO_3^-–N, whereas when wheat-sowing time was delayed under the condition where temperature was low and NH_4^+–N could not be rapidly nitrified, NH_4^+–N had a lesser effect, even a detrimental effect on wheat growth compared with that of NO_3^- treatment (Table 5.6). Owing to such good functions of NO_3^-–N, it is not surprising to get a higher yield from NO_3^-–N than from NH_4^+–N.

Vegetables are crops that strongly prefer NO_3^-–N to NH_4^+–N, and accumulate large amounts of NO_3^- in their tissue (Ai et al., 2000a, 2000b; Crawford and Glass, 1998; Gruda, 2005; Santamaria, 2006; Wang and Li, 2004; Wang et al., 2004a). In fields, due to the rapid transformation of NH_4^+–N or urea N into NO_3^-–N, the two types of N fertilizers often show no difference on vegetable yields, and neither has exhibited a detrimental effect. However, in solution culture, the addition of NO_3^-–N as the sole N source without urea or NH_4^+–N ensures good vegetable growth and has no any detrimental effect on plant growth, while NH_4^+–N exhibits a detrimental effect on vegetable growth. In a solution with only NO_3^- as the N source, the vegetables grew very well while with NH_4^+ as the sole source, the vegetables grew poorly, and even died (Tian et al., 2002, 2003). It has been reported that tomato, radish, and cabbage (common head cabbage) (*Brassica oleracea*) grew very well in high yield with the supply of NO_3^-–N, and the supply of a certain ratio of NO_3^-–N to the NH_4^+–N solution does not only promote the growth and development of vegetable crops but it also declines the NO_3^-–N contents in plants (Demsar and Osvald, 2003; Serio et al., 2004). Zhu and Jiang (1994) claimed that the optimal ratio of NH_4^+–N to NO_3^-–N concentration in solution for solid headed Chinese cabbage (*B. rapa*, subspecies *pekinensis* and *chinensis*) was 1:1. A water culture experiment from Jiang (1990) showed that compared to NO_3^-–N nutritional solution, roots of Chinese bellflower or balloon flower (*Platycodon grandiflorus*) grown in NH_4^+ solution were poorly developed, absorbing roots were present in a small amount, and root biomass was reduced.

Dam Kofoed et al. (1967) revealed that sugar beet responded better to NO_3^-–N than to NH_4^+–N at high N rates. Recently, some results show that NO_3^-–N does not only increase sugar beet yield, but also its sugar content, so this crop is treated as NO_3^-–preferring plant (C.D. Li et al., 2003; C.F. Li et al., 2003; Zhang et al., 2008). Tobacco is a crop that needs NO_3^-–N, and application of NO_3^-–N has improved its yield and quality (Cao et al., 1991a, 1991b).

Rice grows in paddy soil under a waterlogged condition. It has been believed that rice preferred NH_4^+–N rather than NO_3^-–N (Dai et al., 2001). However, experimental results have shown that rice also does well with NO_3^-–N, and supplement of some NO_3^-–N at a late growing stage significantly promotes rice growth and gives a higher yield than without it (He et al., 1998a; Sasakawa and Yamamoto, 1978; Yang and Sun, 1990). Water culture of rice by Tanaka et al. (1990) showed that when evaluated based on N uptake and grain yield, NH_4^+–N and NO_3^-–N had a similar

effect when both doses were medium (20 mg L^{-1} N), while when the dose was high (100 mg L^{-1} N), NO_3^-–N was better than NH_4^+–N, since NH_4^+–N inhibited root growth and affected N assimilation in leaf blades. As a result, with an increase in concentration, the advantages of the addition of NO_3^-–N compared to the disadvantages of the addition of NH_4^+–N become more obvious.

The magnitude of absorbed and utilized rate of NH_4^+–N and NO_3^-–N by crops mainly depended on crop types (Ismunadji and Dijkshoorn, 1971; Kirkby, 1967, 1981), environmental conditions, especially medium pH, and the external concentration of both N (Gerendas and Sattelmacher, 1990; Smith and Wisshire, 1975). Medium pH affects N uptake amounts of the two forms and therefore their effect on crop yield. For the same crops, the uptake amount of NO_3^- or NH_4^+–N is dependent on medium pH. Low pH value does not benefit the application of NH_4^+–N, and when the pH is >7, the application of NH_4^+–N increases free NH_3 in media and thus has a detrimental effect. In contrast, plants that prefer calcium and that adapt to calcareous soils with high pH use NO_3^-–N as priority (Kirkby, 1967). Some results showed that the relative absorption rate of maize to NH_4^+–N and NO_3^-–N was determined by the age of a plant: seedlings took up NH_4^+–N more rapidly than NO_3^-–N, and thereafter, NO_3^-–N was the major form for plant uptake, generally occupying 90% of the total N amount absorbed. Due to a high pH buffering capacity in dryland soils, the effect of NO_3^-–N and NH_4^+–N on crop yield is different in fields from that in water culture.

Li et al. (1997) top-dressed NH_4^+–N fertilizer, $(NH_4)_2SO_4$, and NO_3^-–N fertilizer, $Ca(NO_3)_2$, to 4-year-aged apple trees (*Malus pumila*) in fields and found that both the N fertilizers could increase the superoxide dismutase (SOD) and peroxidase (peroxide enzyme) (POD) activity to a certain extent, and NO_3^-–N was more striking. With NO_3^-–N rate increase, the activity of the two enzymes was correspondingly increased, but high NH_4^+–N levels, the POD activity was decreased, *Vallisneria natans*, a submerged plant, completely grows in water, and its physiological activities totally depend on water environments. Zhu et al. (2005) studied the physiological effect of different ratios of NO_3^-–N to urea N on the growth of *V. natans*, using water culture experiments under nutrient enrichment conditions (4 mg N L^{-1} and 0.2 mg P L^{-1}). Results show that when NO_3^-–N was only used as the N source, the biomass, soluble sugar contents, and NRA were all higher, while POD was lower than in any other treatment including in the control (without N addition). In contrast, when urea content

gradually increased, a certain stress occurred for its growth; the threshold values for forming stress to the plant was 1 to 1 of NO_3^-–N to urea N. The addition of a certain amount of NO_3^-–N promoted the growth while the gradual increase of urea content in the environment produced a certain stress and affected its normal growth. Other researchers also found that urea decreased plant biomass (Hou et al., 2004). Elena (1996) claimed that the high NH_4^+–N concentration produced by the hydrolysis of urea inhibited the synthesis of urease.

The problem associated with the use of NO_3^-–N by plants is NO_3^- accumulation, and this may be harmful to humans and animals that consume these plants. Graminaceous pastures have no ability to fix N, and the N they need is mainly taken up by roots from the soil. Excessive N fertilization does not only decrease the yield and quality (Che et al., 1987), but it also brings about NO_3^-–N accumulation in plants. Zhang et al. (2007) investigated the relation of NO_3^-–N contents in hybrid Sudan grass to its yield. Their results show that although N addition increased its NO_3^-–N accumulation, especially in stems rather than in leaves, the raw proteins and yield were also increased. Urea, as an organic N compound, can supply carbon and N to plants, and thus become a specific fertilizer for some plants (Reinbothe and Mothes, 1962) growing in water (ponds, pools, and lakes) for raising animals (Fan et al., 2003; Zhang et al., 2004a). However, this has resulted in large amounts of N loss and water pollution (Gao et al., 2002; Xu et al., 2002a).

5.1.3. NH_4^+–N and NO_3^-–N have the Same Effect

Cooke (1978) compared the effect of NO_3^- and NH_4^+ fertilizers in a large number of field experiments and found that in slightly acid soil, the difference in the two N sources was small in most cases of crop growth, and it was difficult to say whether one is superior to the other. Field experiments from Dam Kofoed et al. (1967) with different N rates revealed that, only sugar beet responded better to NO_3^-–N than to NH_4^+–N at high N rates. As a whole, the two N forms had no significant difference on crop yields. Water culture experiments from Schrader et al. (1972) showed that NH_4^+–N and NO_3^-–N were similar in maize uptake quantity when both coexisted. Arnon (1937) and Beevers (1976) revealed that NH_4^+–N and NO_3^-–N were equally effective to plants. Warncke and Barber (1973) adopted water culture in an artificial climatic chamber to study the absorbing velocity of NH_4^+–N and NO_3^-–N with maize seedlings of 13 days. The experiment was conducted 5 days with 5 ratios of NH_4^+ ion to NO_3^- ion from

8.40 to 0.17, and the nutritional pH was controlled at 5.8 or so. Results showed that the ratios in solution varied with N concentrations. When N concentration increased, the ratio trended to 1, and maize seedlings had almost no difference in the relatively absorbed amount of NH_4^+–N and NO_3^-–N. Water culture from Mengel (1983) indicated that when NH_4^+–N and NO_3^-–N coexisted, rice took almost the same amount of both NH_4^+–N and NO_3^-–N, and in cultivated fields, the difference in the two forms of N fertilizer was not significant in crop yields. Zuo and Wei (1983) found from a ^{15}N-labeled experiment in calcareous soil that there was no significant difference in winter wheat yield and N uptake amount on the application of equal amounts N of NH_4^+ or NO_3^-. Reddy and Reddy (1993) indicated that maize grain yields and ^{15}N accumulated amounts in plants were not significant for the two N sources, while the N loss from NO_3^-–N fertilizer was higher than from the NH_4^+–N fertilizer. Wang et al. (1990) revealed that 8-day-old wheat plants had a similar Vmax and Km for both NH_4^+–N and NO_3^-–N.

All these are in agreement with the early results in the 1930s to 1940s of the twentieth century. At that time, Arnon (1937) concluded that either NH_4^+–N or NO_3^-–N could be used as a good N source for plant growth and to obtain high yields. Prianishnikov (1945) obtained the same conclusion as did Arnon. Some research results show that once suitable measures are taken to overcome the accompanying properties, the two N forms have the same effect. In summary of the results pertaining to the effect of N forms on plants, Hageman (1980) reviewed the studied results of NO_3^-–N and NH_4^+–N on plant growth, and agreed with the viewpoint of Arnon (1937) that both NH_4^+–N and NO_3^-–N were good N sources for plant growth and production with NO_3^-–N was more secure.

5.1.4. Combinative Use of the Two N Sources is Superior to that of Either NH_4^+–N or NO_3^-–N Alone

For many years, researchers have mainly compared the effect of NH_4^+–N and NO_3^-–N alone on crop growth and yield. Due to the differences in experimental conditions and crops, some results show that one form of N is better than the other, and others show no difference in crop yield between the two N forms. Because NO_3^- is more easily lost than NH_4^+–N is, and the distribution of soil NO_3^-–N obviously varies with season and space and because the content changes affect plant N, it is difficult to evaluate the effects of the two N forms, and thus no consistent result has been obtained (Hageman, 1984; Lu, 1994; Wang et al., 1990). However, although different

plants may have different sensitivities to NH_4^+–N and NO_3^-–N and plants may have evolved multiple ways for obtaining N, including absorption, fixation, and phagocytosis in adaptation to various environments (Crawford and Glass, 1998), earlier and in recent years, numerous results have shown that the application of NH_4^+–N and NO_3^-–N together in equimolar concentrations has much more favorable effects on plant growth and development, biomass production (Hageman, 1984; He, 1993; Li and Wang, 1993; Lu, 1994; Reddy and Reddy, 1993; Wang et al., 1990; Warncke and Barber, 1973; Zhang et al., 1990), agricultural product quality, as well as on crop yield than the application of either alone (Gazzarrini et al., 1999). In most cases, application of both can reduce costs and protect the environments (H.R. Dong et al., 2004; Guo et al., 2006; Tabatabaei et al., 2006). Also, for the majority of plant species, a mixture of NO_3^- plus some NH_4^+ produces the maximum growth (Michael et al., 1970; Ta and Ohira 1981). Simultaneous supply of the two N forms can regulate cell pH, store part of N in consumption of less energy, and obtain maximum growth velocity. The ratio of the two N forms mainly depends on the total N concentration. Although higher levels of NH_4^+ added to the culture solution can characteristically produce toxic reactions and reduce growth rate and decrease the intake of NO_3^-, supply of NH_4^+ (in small amounts) to the NO_3^- system rather than either alone can give higher plant protein contents (Weissman, 1964) and increase the yield for many plant species (Cox and Reisenauer, 1973; Joiner and Knoop, 1969; Precheur and Maynard, 1983; Van den Driessche, 1971). At sufficiently low levels, the decrease in NO_3^- uptake by NH_4^+ is much less compared to the rapid uptake of NH_4^+. As a direct result, the total intake of N, plant protein content and growth rate are increased. Such results can be expected since nitrification occurs in most fertile soils, and the major form of N is NO_3^- though small quantities of NH_4^+ are usually present. Reisenauer et al. (1982) found that the maximum yields of ryegrass (*Lolium perenne* L.) were obtained from low levels of NH_4^+ (36 μM) with adequate NO_3^- (72 μM). Haynes (1986) reported that most plants had the highest biomass when both NO_3^-–N and NH_4^+–N were simultaneously supplied. Mengel and Kirkby (1987) pointed out that when NH_4^+–N and NO_3^-–N coexisted in the nutritional solution, the N uptake rate was the highest.

The optimum ratio of NH_4^+–N to NO_3^-–N probably differs for different species and may change with plant growing stages and plant age (Michael et al., 1970; Ta and Ohira, 1981). Of the crops, wheat has been regarded as a typical example (Cox and Reisenauer, 1973; Shaviv et al.,

1990). Reisenauer (1978) found that when NH_4^+ and NO_3^- coexisted, the biomass of wheat seedlings was higher compared to that of NO_3^- alone. Cox and Reisenauer (1973) found that wheat yield was the highest when NH_4^+–N and NO_3^-–N were supplied simultaneously. Shaviv et al. (1990) reported that mixed application of NH_4^+–N and NO_3^-–N did not only increase wheat yield but also its tolerance to salinity. It is claimed that in a clay–loam soil, maize growth was the best by application of NH_4^+–N and NO_3^-–N together.

The exact reason for the superiority of NO_3^- plus NH_4^+ over NO_3^- alone is not yet clear. Some workers have ascribed it to the reduced energy requirement in using NH_4^+ instead of NO_3^- or in protein synthesis (Cox and Reisenauer, 1973), and others, in contrast, have related it to the inability of the NO_3^- reducing system to supply the plant with maximum usable levels of reduced N (Reisenauer et al., 1982). Since in plants transferring of nutrients from the roots to the upper part is completed mainly through the xylem, the nutrient composition in the xylem sap can reflect the nutrient supply status from the roots to the upper part. The fact that the amounts of the reduced N in the xylem sap are generally higher in plants fed with NO_3^- plus NH_4^+ than with NO_3 alone (Pate, 1975, 1980) provides evidence of their cosupplying advantage. As a whole, the two N forms have their own properties, and their combinative use can take each advantage and avoid each disadvantage. The addition of either alone can induce media pH changes and produce unbalanced absorption of cations and anions. The N forms have a strong impact on the uptake of other cations and anions, on cellular pH regulation and on rhizosphere pH. The assimilation of NH_4^+ in roots produces about one proton per molecule of NH_4^+ (Raven and Smith, 1976). The protons generated are to a large extent excreted into the external medium to maintain cellular pH and electroneutrality for compensating for the excess uptake of cation equivalents over anion equivalents with NH_4^+–N. Consequently, the rhizosphere chemistry is affected by the form of N taken up: NH_4^+ supply may reduce rhizosphere pH through a net excretion of protons, whereas NO_3^- supply may increase rhizosphere pH through a net uptake of protons from the rhizosphere (Hinsinger et al., 2003). This will affect the availability of other nutrients such as P and micronutrients. Combinative application of both N forms will play buffer and regulative roles (Cao and Tibbitts, 1993; Gentry and Below, 1993; Raab and Terry, 1994; Xiao et al., 2000), make plants more easily maintain the stability of cell pH, accumulate part N in a small consumption of energy, regulate the absorption of other cations and various enzyme activities, rationally use

C skeletons and alleviate the easily induced NH_3 volatilization or NH_3 toxicity due to NH_4^+–N application alone, and thereby increase fertilizer effects and crop yield. For example, NH_4^+ is predominantly assimilated in the roots, imposing a direct demand for C skeletons, which is reflected in higher activities of PEP carboxylase. Compared to NH_4^+, NO_3^- has the advantage of allowing more flexible distribution of assimilation between roots and shoots, and can be stored in higher amounts than NH_4^+ in the vacuoles (Hawkesford et al., 2011). Further, under mixed N nutrition, the proton generated by NH_4^+ assimilation can be used for NO_3^- reduction, and be easier for plants to regulate intercellular pH when both forms of N are supplied.

The combinative ratio of NH_4^+–N to NO_3^-–N has been seldom studied, and reports only concerned a few crops. Zhang et al. (1990) used water culture to study the effect of the combinative ratios of NH_4^+–N to NO_3^-–N on spinach and found that different ratios had greatly different effects on its growth and quality, the biomass, NO_3^-–N content, and oxalic acid content were higher while the total soluble sugar and vitamin C lower for NO_3^- treatment than for NH_4^+ and urea treatments. Considering yield and quality, the optimum ratio of NH_4^+–N to NO_3^-–N was from 1:1 to 3:7 for spinach (Zhang et al., 1990), 1:3 for tobacco, and 1:1 for tomato. Ma and Shi (1986) claimed that under water and pot culture conditions, the biomass of spinach, tomato, onion (*Allium cepa*), and French bean or green bean (*P. vulgaris*) increased with the NO_3^- increase in solution, while in fields, such a phenomenon was not observed.

Zhang et al. (2004b) investigated the responses of 40 rice cultivars to NH_4^+–N and NO_3^-–N with three NH_4^+–N to NO_3^-–N ratios (100:0, 50:50, and 0:100). The results show that all the rice cultivars had good responses to and the highest N uptake, root and plant biomass in the mixed NH_4^+ and NO_3^- solution. Based on the responsive magnitude to the enhancement of NO_3^-–N nutrition, they classified the rice cultivars into high, medium, and low (NO_3^-–N) responding types using N cumulative amount, total dry matter, and root dry matter as main physiological criteria that were considered as the most suitable evaluation parameters. The high NO_3^-–N responding type was higher in all the parameters while the low NO_3^-–N responding type was all lower in these aspects than NH_4^+–N nutrition alone.

Duan et al. (2005a, 2005b) studied the optimum ratio of NH_4^+–N to NO_3^-–N for the best growth of rice. They used eight rice cultivars and five ratios (100:0, 75:25, 50:50, 25:75, and 0:100). Their results showed that ratios

of 75:25 and 50:50 were the best composition for all genotype growth and N uptake. Qian et al. (2003) also showed that the ratio of 75:25 was the best combination for the growth of other rice genotypes.

Preference to NH_4^+–N or NO_3^-–N also relates to crop variety or genotypes, growing stages, crop resistance to acidity or alkalinity, and NH_3–toxicity, the NRA in plants, storage of carbohydrate, root metabolism, and the like. Different cultivars have greatly different responses to NH_4^+–N and NO_3^-–N (Batigar and Barber, 1979). Yang and Sun (1991a, 1991b) reported that the absorbed velocity of NH_4^+–N and NO_3^-–N was higher for hybrid rice than for nonhybrid rice, and the hybrid rice responded better to NO_3^-–N at the late growing stage than did the nonhybrid cultivars.

Plant growing age affects the responses of plants to the two N forms. Tomato is a typical crop that prefers NH_4^+–N at early growing stages due to the reductive process dominating, while it prefers NO_3^-–N at late stages due to the oxidized process dominating (Guo et al., 1999a). Maize takes up more NH_4^+–N at the seedling stage, while more NO_3^-–N at later stages (Hewitt and Smith, 1975; Michael et al., 1970). Bennett (1974) found that in water culture solution, the use of NH_4^+–N was unfavorable to 9-week-old maize. Yang and Sun (1986, 1990) showed that rice predominantly took up NH_4^+–N at vegetative growing stages, while it took up NO_3^-–N at the reproductive stage. Wen (1993) indicated that during the entire-growing period of rice, application of NH_4^+–N was better than NO_3^-–N. However, application of NH_4^+–N at early stages and NO_3^-–N at late stages or alternative use of NH_4^+–N and NO_3^-–N obtained higher yields than NH_4^+–N did alone.

From the results mentioned, we can see that a large number of studies have been conducted from different aspects such as uptake amount, uptake ratio, and the velocity of NH_4^+–N and NO_3^-–N, but most of the work used water culture with limited crop species. These results need further confirmation by more experiments and more crop plant species.

5.2. Enhancement of NH_4^+–N

For many dryland crops such as wheat and cotton, due to nitrification being conducted rapidly in soil, plants are mainly nourished by NO_3^-–N nutrition, and maintenance of NH_4^+–N in soil has not been given full attention (Crawford and Chalk, 1993). Research studies have shown that when the mixed nutrition of NH_4^+–N and NO_3^-–N coexists in media, many crops have higher dry matter production, N uptake, and N use efficiency compared to either alone (Below and Gentry, 1992; Below and Heberer, 1990;

Camberato and Bock, 1990; Cao and Tibbitts, 1993; Cox and Reisenauer, 1973; Dai et al., 1998, 2001; Heberer and Below, 1989). Based on this fact, a concept, enhancement of ammonium N nutrition (EANN), has been proposed and received great attention (Gentry and Below, 1993; Heberer and Below, 1989). This is to increase the NH_4^+–N concentration in soil, particularly in the rhizosphere under the condition of NO_3^-–N being predominant. This measure has been proposed to increase N fertilizer efficiency for different crops (Dai and Cao, 2002; Dai et al., 1998, 2001; Raab and Terry, 1994). Li (2002) conducted a pot experiment to study maize responses to the EANN. Two maize cultivars were used and different ratios of NH_4^+–N to NO_3^-–N supplied. The results show that the highest dry matter and per plant yield were obtained in the ratio of 1:2 for one cultivar and 2:1 for the other. Compared to either NO_3^-–N or NH_4^+–N alone, the EANN significantly increased chlorophyll contents, photosynthetic velocity, NRA, cumulative N amount, dry matter production, and yield. An experiment from Dai and Cao (2002) show that leaf area increase is the main cause for the EANN to improve maize plant growth. Other results indicate that much more N uptake by the EANN than either is the main cause for high dry matter production (Wang, 1992). Dong et al. (2001) adopted a water culture experiment to evaluate cotton cultivar responses to three ratios of NH_4^+–N to NO_3^-–N nutrition (0:100, 50:50, and 100:0) in root morphology and plant growth. The results showed that each of the two N forms had its own advantages. For all cotton varieties tested, NO_3^-–N was beneficial to cotton shoot growth whereby NH_4^+–N was beneficial to root formation. However, as a whole, the EANN with a 1:1 ratio of the two N forms had the best results for cotton seedling growth in increase of leaf areas, tap root length, number of the first lateral roots, leaf photosynthesis, and carbon assimilation as well as a significant increase in plant dry matter compared to either alone. Bubán et al. (1978) and Shear et al. (1953) reported that NH_4^+–N was beneficial to flower formation of fruits trees compared to NO_3^-–N, and the cause was not clear.

5.3. Enhancement of NO_3^-–N to Rice

Great attention has been paid to rice (*Oryza sativa* L.) for the combinative use of NH_4^+–N and NO_3^-–N. Rice is an important food crop in the world and provides 40% heat energy for mankind (De Datta, 1981). As a cereal grain, it is the most important staple food for a large part of the world's human population, especially in Asia and in the West Indies. Rice grain production ranks the third highest worldwide, after maize (corn) and

wheat, according to data for 2009 (FAO, 2009). Its plantation area occupies one-third of the world's total cereal areas (Faferia et al., 1991; FAO, 2009). Since a large portion of maize crops are grown for various purposes other than for human consumption, rice is the most important grain with regard to human nutrition and caloric intake, providing more than one-fifth of the calories consumed worldwide by the human species (Smith, 1998). With rice yield increase, the rate of chemical fertilizers used, particularly N fertilizers, is continuously increasing. The N rate used for a single cropping of rice is too high, and the N use efficiency is too low, especially in China (Peng et al., 2002). Overapplication of N fertilizer to rice has led to a series of environmental problems (Li and Li, 2001; Wang and Li, 2004). For increase of the N efficiency of rice, the enhancement of nitrate N nutrition (ENNN) to rice or addition of NO_3^-–N to rice growing media in which NH_4^+–N is predominant has been proposed and is of great concern to many scientists. Such a measure has significantly improved rice productivity and has increased its yield.

5.3.1. Nitrification Existing in Rice Rhizosphere

Rice is in fact nourished by NH_4^+–N and NO_3^-–N, together. Growing in a waterlogged ecological environment, rice growth is characterized in the condition where nitrification is strongly inhibited and NH_4^+ concentration is remarkably high, becoming the major form of N source in the paddy soil. For this reason, rice has been regarded as a typical plant preferring NH_4^+–N (Dai et al., 2001; Sasakawa and Yamamoto, 1978). As a result, NH_4^+–N nutrition has been emphasized, while NO_3^-–N nutrition ignored. However, long ago, agricultural workers found the occurrence of nitrification in rice rhizosphere. Even under waterlogging conditions, rice can transport oxygen (O_2) through aerenchyma from plant stems to roots (Armstrong, 1971; Blom and Voesenek, 1996; Justin and Armstrong, 1987), and secrete or release part of O_2 into rhizosphere soil (Frenzel et al., 1992). Such oxygen can be used by bacteria conducting nitrification in the soil, and it stimulates the growth and reproduction of nitrifying bacteria in rhizospheres and in root surface areas, and can finally oxidize NH_4^+ into NO_3^-. Therefore, nitrification occurs immediately at the root surface and rhizosphere where NH_4^+–N exists. Revsbech et al. (1999) analyzed the rice rhizosphere using an O_2 microinductorium (sensor) and found that after 3 weeks of rice being transplanted in the field, the O_2 concentration around the roots could reach 20% of the atmosphere when saturated, and meet the living demand of nitrifying bacteria. Consequently, NO_3^-–N in rhizospheres is formed

by nitrification of NH_4^+–N, and therefore, rice in fact thrives when nourished by NH_4^+–N and NO_3^-–N together even under completely waterlogged conditions. Li et al. (2006) found that the nitrification intensity in rhizospheres of two rice cultivars was increased with the progress of rice growth, and this might relate to the increase of oxygen-secreting ability that increased with the progress of plant growth (Ghosh and Kashyap, 2003). Increase of oxygen concentration around the roots correspondingly increased the NH_4^+-oxidizing bacteria and further provided the substrate for nitrification. Consequently, the nitrification intensity further increased.

The nitrification has a space variation in rice field in relation to either the NH_4^+ content or the oxygen amount released from the roots. If serious deficiency of NH_4^+–N occurs in rhizospheres, nitrification intensity may be lower than in bulk soil. After NH_4^+–N is absorbed by plant roots, the pH value on the root surface is rapidly decreased (Colmer and Bloom, 1998), and following this, nitrification intensity is decreased. In general, nitrification intensity in rhizospheres is much greater than in bulk soil and the intensity has great variation. The highest intensity is believed to be caused by the strongest secretion of oxygen in root tips, and the variations in different areas to be induced by the different ability for secretion of oxygen (Ghosh and Kashyap, 2003). The secreted oxygen amount was decreased with the distance from root tips, and the minimum amount was found at a 30- to 60-mm distance from root tips (Armstrong, 1971). Li et al. (2006) found that the rhizosphere nitrification intensity was different for different rice cultivars, and the highest nitrification intensity occurred at a 6-mm distance from the root tips for one cultivar while at a 4-mm distance for the other.

Experiments from Kirk (2001) revealed that nitrification became weaker for longer distances from the roots. This may be due to two reasons: first, the nearer the rhizosphere, the higher the oxygen concentration, and the higher the nitrification. Second, during the growing period of rice roots, a large number of organic substances may be released, and this may lead to a different distribution of nitrifying bacteria: the nearer it is to the rhizosphere, the larger the number of nitrifying bacteria, and the higher the activity. By using fluorescence, Aurelio et al. (2003) observed using fluorescence in situ hybridization that the nitrifying bacteria around roots formed discontinuous biological films that surrounded the rice root surface. The nitrifying bacterium density in such "biological films" was 10^7 cells cm^{-3} of root, 100–1000 times higher than that of nonrhizospheres. The existence of a large number of reactively nitrifying bacteria

shows that a large part of N absorbed by rice is NO_3^-–N formed by nitrifying organisms. The oxygen-released characteristics of rice roots can make soil nitrifiers oxidize large amounts of NH_4^+ into NO_3^-, and thus cause rice to absorb NH_4^+ and NO_3^-–N and increase growth and development. Therefore, nitrification plays a decisive role for NH_4^+ and NO_3 levels in rhizosphere soil. Kirk and Kronzucker (2005) show by a model calculation that under field conditions, the rhizosphere of waterlogged plants can form a large amount of NO_3^-–N through nitrification, and the NO_3^- formed can be rapidly absorbed and used by rice roots; the uptake velocity even can be compared with that of NH_4^+. Due to instant absorption of NO_3^-–N formed on rice root surface and rhizosphere by rice roots, the NO_3^-–N amount is too small to be detected in soil samples taken from paddy soil (Duan et al., 2004) or the detected amount is small. From these results, it is clear that even in complete waterlogged conditions, the root oxygen-secreting characteristics of rice can be sufficient for significantly increasing the nitrifying bacteria in soil, and these bacteria can oxidize large amounts of NH_4^+ into NO_3^-, leading to the existence of a significant amount of NO_3^-–N in the rhizosphere. As a result, rice actually exists in a mixed solution of NH_4^+–N and NO_3^-–N or in the "enhancement of NO_3^-–N nutrition."

Rice can take up and use NO_3^-–N, and the beneficial effect of NO_3^-–N on rice has long been realized. In the 1960s to the 1970s of the twentieth century, scientists discovered that rice took up and used NO_3^-–N as easily as did NH_4^+–N (Fried et al., 1965; Sasakawa and Yamamoto, 1978). Compared to sole NH_4^+ nutrition, the NH_4^+ and NO_3^- mixed nutrition can make rice obtain a larger biomass and economic yield, and higher N recovery rates (Weissman, 1964). Such reports have been continuously increased since then (Kronzucker et al., 1999; Ta and Ohira, 1981; Zhang et al., 2004b). Raman et al. (1995) reported that rice roots could absorb NO_3^-, and its leaves could reduce NO_3^-. Wang et al. (2003) found that different rice cultivars had different characteristics of uptake kinetics of NO_3^-–N and thus had significantly different abilities in uptake of NO_3^-. Kronzucker et al. (1999) used a series of low concentrations of NO_3^-–N (2.5–500 μM) and found a high-affinity transport system of NO_3^-–N in rice with a *K*m value of 26 ± 5.6 μM. Fan et al. (2005a) in study of NO_3^- absorption kinetics show that two NO_3^- absorption systems, high affinity and low affinity, exist in root cell membranes of rice, indicating that rice can absorb and use NO_3^-–N just like crops do when grown in dryland areas. Studies from Duan et al. (2007) show that rice cultivars with different N efficiencies can

all absorb and use NO_3^-, and the maximum NO_3^-–N uptake amount can reach 44% of the total absorbed N.

5.3.2. Promotion of Root Growth

The ENNN has a good effect on rice root growth. It has been revealed that there is a relation of NO_3^-–N in soil to root growth and development. Root dry weigh is a very important index for the evaluation of rice responses to NO_3^-. An accepted reason for the higher yield of rice with seedlings cultured in dry conditions than that cultured in wet conditions is that the former has more root number, is more fully developed roots, and has a higher abundant root biomass. The lateral roots grown under NO_3^- nutrition are better than those deficient in NO_3^-. Zhang et al. (2004b) found that the ENNN (by adding NO_3^-–N to the solution to make the total N concentration to 2.86 mmol L^{-1}), to rice at the eight leaf stage had produced more lateral roots, and the root dry weight was 20–40% higher than that nourished by NH_4^+ nutrition alone at the same time. Noma et al. (2006) found that cultured with NO_3^-–N nutrition, rice lateral root number and volume were significantly higher than NH_4^+ nutrition. Different cultivars of rice had great differences in responses to NO_3^-–N enhancement. A splitting root experiment proves that with the addition of NO_3^-–N, the lateral roots are much better than that without NO_3^-–N (Wang et al., 2002a). Another root-split experiment found that roots immersed half in NH_4^+ solution and half in NO_3^- solution were more significantly increased than those in NH_4^+–N solution, and were close to those (roots) cultured in the mixed solution with both. However, such results might not be the direct function of NO_3^-–N, since NO_3^-–N was not detected from the beginning to the end in the NH_4^+ side solution that nourished part of the rice roots. At present, for an understanding of the mechanism of NO_3^-–N in the stimulation of the lateral roots, the majority of the workers have focused on *A. thaliana*, maize, and barley. The results suggest that the existence of NO_3^- concentration <10 mmol L^{-1} (Zhang et al., 1999) might promote the formation and transportation (long distance and short distance) of signal materials (Forde, 2002) that in turn stimulated root growth. Such results may be helpful to further study the stimulating mechanism of NO_3^-–N to rice lateral roots.

Carbohydrates are important signal substances for regulating plant growth, development, and metabolism (Smeekens, 2000). No matter whether carbohydrates are obtained by external application or by phloem transport, they all can stimulate lateral root growth and development (Bingham et al., 1998; Crookshanks et al., 1998). Scheible et al. (1997) found

in tobacco studies that the transport of carbohydrates between shoots and roots affected root growth. When plants were deficient in NO_3^-, the carbohydrate contents in shoots greatly increased, and the ratio of roots to shoots decreased. Therefore, NO_3^- may regulate the transport of carbohydrates between roots and shoots to influence lateral root growth. However, some workers obtained opposite results (Walch-Liu et al., 2001), and attributed such a function of NO_3^-–N in the promotion of rice root growth to the rapid synthesis and transport of root endogenous hormones, mainly CTK and auxin indole-3-acetic acid (IAA) (Revsbech et al., 1999). This will be discussed in more detail in a later section.

Most workers have accepted the view that NH_4^+–N can inhibit the root growth of rice, but the inhibitive mechanism has not yet been made clear. At early stages, researchers postulate that the effect of NH_4^+–N inhibition on plant root growth was due to carbon level limitation. However, many experiments (Chaillou et al., 1986; Kandlbinder et al., 1997; Walch-Liu et al., 2001) indicate that under NH_4^+–N nutrition conditions, the carbohydrates were increased in both roots and shoots. Thus, carbohydrates could not have been the limiting factor for root growth.

To sum up, there exist four views on the ENNN in stimulation of the lateral root growth of rice: (1) nutrition function: the NO_3^-–N provides plant N nutrients, and thereby stimulates lateral root growth (Hackett, 1972); (2) stimulative function: NO_3^-–N is considered a root-stimulative chemical in promoting root growth. In a root-split experiment of *A. thaliana*, the supply of NO_3^-–N stimulated lateral root growth, while the supply of the same amount of other N sources such as NH_4^+–N or glutamic acid (Glu) salt did not have such effects (Zhang et al., 1999). This shows that the main function that NO_3^- plays may not be a nutritional but a simulative role, and this role cannot be replaced by other N sources; (3) stimulation of hormone formation: some results show that NO_3^-–N may have a function in the rapid increase of the synthesis and transport of the root-produced hormone CTK and auxin IAA that in turn can stimulate root growth (Walch-Liu et al., 2001, 2000); (4) signal assumption: NO_3^-–N under extremely low concentrations may act as a signal substance to stimulate lateral root growth.

5.3.3. Effect of ENNN on N Assimilation and Utilization

Addition of NO_3^-–N obviously affects the uptake and use NH_4^+–N of rice. Duan et al. (2004) found that with the ENNN, the *V*max of NH_4^+–N was increased by 26–39%, while the *K*m was only increased by 1.06–9.55%. This clearly indicated that the ENNN significantly increased NH_4^+-transport

protein expression while having no great influence on the affinity of the absorbing site to NH_4^+–N. Therefore, the function of NO_3^-–N in the improvement of NH_4^+–N uptake mainly depends on increasing the transport velocity of NH_4^+ rather than on increasing the affinity. A ^{15}N–labeled experiment from Duan et al. (2007) showed that compared to NH_4^+–N nutrition, the ENNN increased the net uptake of $^{15}NH_4^+$ by 13% and 10% in rice leaves and 23^+ and 27^+ in rice roots at seedling and peak tillering stages, respectively. That is, the ENNN does not only promote rice root growth but also root uptake of NH_4^+–N efficiency. This significantly expands plant N sink and further enhances the rice grain yield potential.

A ^{13}N-labeled experiment using rice IR72 showed that the existence of NO_3^-–N could promote uptake, accumulation, transport of NH_4^+ in plasma membrane, and metabolism of NH_4^+ in rice cells (Kronzucker et al., 1999). Kronzucker et al. (2000) further found that with the ENNN, the influx amount of NH_4^+ into cells was increased 25% and that the efflux amount was reduced by half compared to that for NH_4^+–N. This means that the net cell uptake of NH_4^+ was increased by 50%. In contrast, the NH_4^+ content in the cytoplasm was not significantly changed. They also found that with the addition of NO_3^-–N, 56% of NH_4^+–N taken up by rice plants could transport to the upper part, while only 26% when NH_4^+ was the sole N source. Analysis of microarrays shows that changes in mineral N forms can affect the amounts of transport proteins as much as to twofold for three high NH_3-affinity and two low NH_3-affinity varieties of rice. When the N level supplied is 0.2 mM, the coexistence of NH_4^+ and NO_3^- has increased the net uptake velocity by 20–75% compared to NH_4^+–N existence alone. When the external supply of N outstrips 0.2 mM (the available N in waterlogged rice field generally ranges from 0.1 mM to 5 mM), the net uptake velocity further increases until the net uptake velocity reaches saturation (Kronzucker et al., 1995, 1996; Siddiqi et al., 1990).

The role of NO_3^- in promoting NH_4^+ use may be exhibited in different ways. In addition to promoting NH_4^+–N uptake, it may, as one way, promote NH_4^+–N assimilation in rice plants. Assimilation of NO_3^-–N in either roots or shoots of rice will promote the absorption of NH_4^+–N by roots. Since >95% NH_3 in plants is assimilated by GS as the first assimilative step (Lea and Miflin, 1974), the GS activity can be used as a better index for characterizing root synthesis (assimilation) of NH_3 (Lea et al., 1992). Experimental results have shown that only under the existence of NO_3^- conditions (Redinbaugh and Campbell, 1993) does the GS–GOGAT system in rice root proplastid work actively, and it makes the absorbed NH_4^+ rapidly

assimilated and used by plants, and then further enhances the N uptake of rice and transport to shoots. Zheng (1986) reported that the simultaneous supply of NH_4^+–N and NO_3^-–N made the GOGAT have the highest activity of rice. Duan et al. (2004, 2005a, 2005b, 2006) found that the ENNN could significantly increase GS activity in rice leaves, and therefore raised the assimilative ability of NH_4^+ of rice. Kronzucker et al. (1999) conducted a molecular level study on the absorption properties of NH_4^+ and NO_3^-–N and found that with the ENNN, the net uptake amount of NH_4^+ into cells was increased by 50%, but the NH_4^+ contents in cytoplasm were not significantly changed. Results from Lam et al. (1996) indicated that NO_3^-–N could induce an increase in GS activity in roots and accumulation of mRNA. Some researchers have shown that in rice GS1 genes, *OsGLN1;1* and *OsGLN1;2*, similar to *GLN1;1* and *GLN1;4* in *A. thaliana*, are high-affinity genes, and a high concentration of NH_4^+ can inhibit their expression (Ishiyama et al., 2004a, 2004b). In nutritional media with NO_3^- existence, the free NH_4^+ concentration is lower than that of NH_4^+alone, and therefore, the GS activity and the assimilation and utilization ability of NH_4^+–N are increased.

Leaves are one of the main sites for NO_3^-–N reduction. The NO_3^-–N absorbed by rice can be partly reduced and assimilated in leaves through NR. Determination of NRA in leaves of rice seedlings showed that (Duan et al., 2005a, 2005b) when NO_3^-–N was present, the NRA in three rice cultivars having different N efficiencies varied from 0.02 to 0.63 NO_2^- μmol g^{-1} h^{-1}, equivalent to that in cotton leaves (0.3–0.5 NO_2^- μmol g^{-1} h^{-1}). Edward et al. (1998) studied NRA in leaves of rice seedlings and found that by the induction of NO_3^-–N, the NRA was increased to a large extent in leaves for all cultivars tested. The presence of NH_4^+–N inhibited leaf NRA for most genotypes of rice cultivars. Based on those studies, some researchers postulate that NO_3^-–N itself is a signal substance for the stimulation of NO_3^-–N assimilation and the relevant genes' expression; NO_3^-–N and other N forms can also partially regulate the expressions of photosynthetic and cell metabolism-relevant genes.

From all results shown above, we can see that the high and stable yield of rice may relate to its existence in the mixed nutrition of NH_4^+–N and NO_3^-–N. Compared to NH_4^+–N nutrition, the ENNN significantly increases rice root growth and root biomass, NH_4^+-uptake velocity (uptake amount of NH_4^+ per unit time and per unit root weight), plant total N uptake, the cumulative capacity of N per plant for rice cultivars that strongly respond to NO_3^-–N, and therefore increases the N use efficiency,

Table 5.7 Fresh Weight of Rice Seedlings (g/56 plants)

Treatment	Roots	Shoots	Total
NH_4^+ + aeration	168	350	518
½NO_3^- + ½NH_4^+ + aeration	234	444	678
NH_4^+ + nonaeration	222	432	654
½NO_3^- + ½NH_4^+ + nonaeration	272	480	752

Malavolta (1954).

and finally its biological yield and grain yield. The ENNN of rice has received great attention for quite a long time. As early as in 1954, Malavolta (1954) found that no matter whether there was aeration or not, the mixed nutrition of both NH_4^+–N and NO_3^-–N could make rice produce more biomass (Table 5.7). Under aerated conditions, the mixed N nutrition increased the total weight of rice by 31% compared to NH_4^+–N nutrition, and under anaerobic conditions, by 15%. Late researchers further proved the advantages of the ENNN in cultured solution or field in the promotion of rice growth compared to sole NH_4^+ nutrition (Anderson et al., 1991; Dai et al., 2001; He et al., 1998a; Marschner, 1995; Smiciklas and Below, 1992). More biological yield and economic yield were obtained and even 40–70% yield increase was reported (Cox and Reisenauer, 1973; Duan et al., 2006; Weissman, 1964), and significant N absorption and N recovery rate increased (Ta et al., 1981; Weissman, 1964). Qian et al. (2004) found that with sole NH_4^+ nutrition, rice yield was 85.2 g pot^{-1} under a solution-cultured condition, while with the ENNN, it was increased to 101 g pot^{-1}.

At present, due to the rapid extension of water-saving cultivation techniques for rice, more attention has been given to NO_3^-–N nutrition properties. Although NO_3^-–N fertilizer has not been considered for use in rice, the ENNN has increased N use efficiency, biological yield, and seed yield of rice. In the future, it may be an important method for obtaining high yields, where rice cultivation is alternated between dry and wet conditions (Yang and Sun, 1992).

5.4. Reasons for the Preference of Crop Plants to the Two N Forms

Although the mechanism by which plants exhibit a preference to NH_4^+ or NO_3^- are not fully clear, many different assumptions have been proposed for explaining the preference. It is now known that the adaptabilities or the

preferences of plants to NH_4^+–N and NO_3^-–N are affected and controlled by external and internal factors.

5.4.1. Plant Characteristics

Plant species, cultivars, and their growing stages are internal factors in relation to plant properties and have been of remarkable concern. Based on the responses of crop plants to NH_4^+–N or NO_3^-–N, some crops such as rice are regarded as NH_4^+-preferring crops, and others such as wheat, maize, tobacco, and pakchoi as NO_3^--preferring crops. It seems that most plants have evolved under conditions of a very low NH_4^+ supply, with NO_3^-–N being the predominant form of available N. These plants are not adapted for use of NH_4^+ as their major or sole N source. In contrast, other plants developed under NH_4^+-fed condition are capable of using NH_4^+ as the main N source. The lack of the ability to effectively reduce a large amount of NO_3^-–N seems to be the most obvious reason for some of these plants to have a preference to NH_4^+–N. It has been reported that many members of the family Ericaceas have significant NRA in their roots (Dirr, 1974), but very low in their leaves even when NO_3^-–N is supplied (Dirr, 1974; Havill et al., 1974; Lee and Stewart, 1978). Roots for many ericaceous plants are the main sites for NO_3^- assimilation, while leaves cannot reduce NO_3^- efficiently. From this point, leaf RNA does not seem important. However, when the root NR system becomes saturated with NO_3^-–N, the leaves cannot act as a major sink for the assimilation of the surplus NO_3^-; hence, these plants cannot efficiently use NO_3^- as their major source of N. This is also true for the calcifuge species (Havill et al., 1974; Osborne and Whittington, 1981; Routley, 1972; Stewart et al., 1974; Townsend, 1966): some possess NRA and have the capacity to reduce NO_3^- in their leaves but some do not due mainly to the significant flushes of NO_3^-–N in soils even in the pH range 3.5–4 (Dary and Taylor, 1974; Taylor et al., 1982).

Carbohydrate amount is regarded as a mechanism for the preference. Some physiological studies show that sugar content is related to NO_3^- uptake (Delhon et al., 1995). The uptake velocity of NO_3^- by soybean is higher in the photoperiod than in the dark period. In the case of removing CO_2 in the atmosphere or implementation of ring cutting of the stem to stop sugar moving to the roots, the stimulation of light to NO_3^- absorption is inhibited while the supply of sugar to the roots removes the inhibition. Plants grown in a lower optical density or in a shorter duration of sunshine contain very high NO_3^- (Matt et al., 1998; Stitt and Krapp, 1999). All this shows that when the carbohydrate content is lower, the NO_3^-–N

assimilation is more inhibited than its uptake, and the energy needed for the absorption of NO_3^-–N is far lower than that for assimilation. Improvement of the photosynthetic conditions will make the NO_3^-–N to be rapidly used.

5.4.2. Chemical Properties of NH_4^+ and NO_3^-

The preferences are also determined by the two ions (NH_4^+ and NO_3^-) chemical properties that affect N loss and N efficiency to plants and the uptake of other anions and cations. Although not noted by the majority of the workers, the behavior of the two N ions, NH_4^+ and NO_3^-, in soil induced by their chemical properties may be an important factor for plant preference. Considering the specific characteristics, NH_4^+ does not contain oxygen, and it is a reduced, positively charged cation. The NH_4^+ can be adsorbed by soil colloids and fixed by both clay minerals and soil organisms. When NH_4^+–N fertilizer or urea is applied to the soil, especially to calcareous soil, it can easily release NH_3 that is subject to volatilization to the atmosphere, a significant process for N loss. Most of the volatilized N will return by wet and dry deposition to lands and waters (Fenn, 1998; Su et al., 2003, 2005). Almost all of the deposited (>95%) NH_4^+–N remains in the soil (Grennfelt and Hultberg, 1986) and plays a role as N fertilization, benefiting plant growth and agricultural production. However, a large amount of deposited N input can lead to various effects on the ecoenvironmental systems of forestry, agriculture and waters (Cuesta-Santos et al., 2001; Matson et al., 2002; Pryor et al., 2001; Salahi et al., 2001; Skeffington, 1990) such as intensification of N saturation in some terrestrial ecosystem, acidification of soil (Bartnicki and Alcamo, 1989), and decline of biodiversity and ecosystem functioning. Overloading of wet deposited NH_4^+ in terrestrial ecosystems and a high rate of NH_4^+ uptake can induce imbalanced uptake of cations by plants. The rhizospheric zone could be quickly acidified, and cations such as K^+, Ca^{2+}, and Mg^{2+} could become inhibited. All this is harmful to plant growth, particularly to forest plants (Matson et al., 2002; Pryor et al., 2001).

In contrast, the NO_3^- is an oxidized, negatively charged, and oxygen-contained anion. In aerobic soils, the applied NH_4^+ fertilizer or urea will be transformed into NO_3^-–N through nitrification (Li et al., 1993). The NO_3^-–N cannot be adsorbed by soil colloids and is readily soluble in water and exists in soil solution. Although NO_3^- can easily move to the root surface for plant uptake and accumulates in plants, it also can be subject to leaching from the topsoil to deep soil layers, and ultimately to groundwater, and can be transported by runoff to surface waters resulting in pollution for ground and surface water (Keeney, 1982; Ma and Shi, 1986) as well as transformed

into N_2O, NO, and N_2, a process known as denitrification. Nitrogen fertilizer and organic fertilizer contribute 55–80% to the total N_2O emission (OECD, 1998). NO_3^-–N leaching is a strong process of acidification and can lower soil pH, change the release and migration of soil base cations and H^+, Al^{3+}, increase Al and Mn activity, decrease soil fertility, reduce tree growth, and damage the forest ecological stability (Bergkvist and Felkeson, 1992).

Since the preference is mainly evaluated by NH_4^+–N or NO_3^-–N influence on crop growth and yield, their behavior in soil must be considered since the loss and utilization efficiency will affect plant growth and yield, and thus the preference. It has been suggested that due to the leaching loss, NO_3^- could show less effect on plants compared to NH_4^+–N (Sun, 1990).

The complementary ions for NO_3^- and NH_4^+ salts are different. Loo (1931) compared the effect of complementary ions of both NH_4^+ and NO_3^- salts on the growth of plant seedlings, and found that the effect of the two N sources was related to the complementary ions. In some cases, the complementary ions could directly affect crop growth, while in some other cases, they only played an indirect function (Hageman, 1984). For example, the application of a large amount of $(NH_4)_2SO_4$ or $NaNO_3$ annually to soil that was low in exchange capacity could result in the soil being acidified or alkalized, and therefore led to the decline of some micronutrient availability and further affected plant growth.

The uptake of NH_4^+ and NO_3^- directly affects the balance of cations and/or anions. Grown on media with NO_3^- as a sole N source, plants generally preferring NH_4^+–N often exhibit an iron deficiency as lime-induced N chlorosis (Cain, 1952, 1954; Nelson and Selby, 1974) although high Fe contents in leaves are sometimes recorded (Cain, 1952, 1954). NO_3^- nutrition can promote cation accumulation to high levels (Cain, 1954; Ingestad, 1973, 1976, 1979), and this in turn results in the production of balancing organic anions in the plant once NO_3^- is reduced (Raven and Smith, 1976). The iron-deficient symptoms produced in plants with NO_3^- nutrition are associated with high concentrations of the organic acid anions (Nelson and Selby, 1974; Su and Miller, 1961; Wallace, 1971) that may bind with Fe in both roots and leaves, thereby interfering with the functions of Fe in the plants (Nelson and Selby, 1974; Wallace, 1971).

Under acidic soil conditions with NH_4^+ being predominant, high concentration of metals such as Al and Mn exists in solution (Foy and Fleming, 1982). Thus, there would be interactions between NH_4^+ and NO_3^- nutrition and Al and Mn toxicities. Ambient NH_4^+ inhibits the plant uptake of Mn and Al, while ambient NO_3^- enhances their uptake (Rorison, 1980). As

a result, NH_4^+ nutrition may, to some extent, be involved in the tolerance of plant to high levels of Al and/or Mn.

5.4.3. Environmental Factors

The external or environmental factors such as soil properties, media pH, types and amounts of anions and cations, NH_4^+ concentration in solution, temperature, illumination or light, and aeration have received great attention for the uptake and use of NH_4^+–N and NO_3^-–N (Barber, 1984a; Hageman, 1984; He et al., 1996; Mengel and Kirkby, 1987; Prianishnikov, 1945; Yang and Sun, 1991a).

5.4.3.1. Temperature

Temperature affects the uptake ratio of NH_4^+–N to NO_3^-–N. At low temperature, any crop takes up more NH_4^+–N than NO_3^-–N (Clarkson and Warner, 1979; Ganmore-Neumann and Kafkafi, 1983; Haynes, 1986; Mengel and Kirkby, 1987). Lycklama (1963) showed that when medium temperature was at 8 °C, lettuce took up more NH_4^+–N than NO_3^-–N; at 25 °C, the NH_4^+–N uptake was the maximum. NO_3^-–N uptake increases with temperature: at 23 °C, its uptake was higher than NH_4^+–N; and from 23 to 35 °C, the uptake amount continuously increased. Grasmanis and Nicholas (1971) found that when the ratio of NH_4^+–N to NO_3^-–N was 1, growing apple trees (*Pyrusmalus*) absorbed NH_4^+–N and NO_3^-–N in the ratio of 1:7. In the winter season, more NH_4^+–N was taken up than NO_3^-–N, since low temperatures led to low velocity absorption of NO_3^-–N. The effect of temperature is related to the pH of the media. When pH in the medium ranges from 4.0 to 6.5, higher temperature benefits NH_4^+–N uptake with the most suitable temperature being 27 °C; when the pH ranges from 6.5 to 8.5, the NH_4^+–N uptake has no relation to temperature. High temperature also benefits the plant uptake of NO_3^-–N with 35 °C being the most suitable temperature for the uptake of NO_3^-–N; when temperature is <13 °C, crops take up almost no NO_3^-–N (Hageman, 1984; Lycklama, 1963). Van den Honert and Hooymans (1955) point out that in the range from 5 to 30 °C, the NO_3^- uptake of maize increases with temperature. Lycklama (1963) shows that in the range of 20–25 °C, ryegrass has the highest velocity for the uptake of NO_3^-–N. Barber (1984a) reports that at 30 °C, maize has the highest velocity of NO_3^-–N, while at 25 °C, sheep fescue (*Festuca ovina*) and (common) reed grass have the highest velocity.

Although the effect of temperature on NH_4^+–N and NO_3^-–N uptake by crops has different results in the literature, the following conclusions are

commonly accepted: (1) temperature effect is related to crop species and medium pH; (2) at low temperatures, the uptake of NH_4^+–N is higher than of NO_3^-–N, whereas at higher temperatures, the uptake of NO_3^-–N is higher than that of NH_4^+–N; (3) the highest velocity for NH_4^+–N occurs at 25–27 °C, while that for NO_3^-–N at 20–35 °C; (4) considering the relation of temperature to the uptake of NH_4^+–N and NO_3^-–N, application of NH_4^+–N should take from early spring and late autumn with a lower temperature while in summer, with a high temperature, the application of NO_3^-–N may be suitable. Clarkson and Warner (1979) postulate that the faster uptake of NH_4^+–N at low temperatures is due to physical changes occurring in different areas of the plasma membrane, but not due to the difference of sensitivity of the two ions to temperature during their transport process.

5.4.3.2. Illumination

Illumination affects the crop uptake of NH_4^+–N and NO_3^-–N from another aspect. Crop uptake of nutrients is an energy-consuming process, and the amount of nutrient uptake must be controlled by the supply of energy. When illumination is sufficient, photosynthetic intensity is strong, abundant energy is supplied, and nutrient uptake is increased. Uptake of NO_3^-–N consumes more energy than NH_4^+–N does, and the effect of illumination on the NO_3^- uptake is more significant. A water culture experiment with rice seedlings conducted by Mengel et al. (1976) show that with dark treatment of 48 h, the NH_4^+–N uptake was reduced by 45%, while NO_3^-–N by 5-fold compared to normal illumination.

It has been proved that the reduction of absorbed NO_3^-–N in plants needs the participation of NR, and the NR activation needs light. If illumination is not enough, the NRA will be low. The accumulation of NO_3^-–N in plants not only affects the further uptake of NO_3^-–N but also product quality. Correspondingly, when the synthesis of carbohydrates reduces, the formation of organic acid will decline, and thereby the assimilation of NH_3 will get retarded. This not only affects the N uptake, but the exceedingly accumulated NH_3 can make plants toxic, and further affects crop uptake of other nutrients (He and Meng, 1987; Mengel, 1983; Ni, 1982).

5.4.3.3. Aeration

The aeration of media can at the same time affect crop absorption of NH_4^+–N and NO_3^-–N. With sufficient oxygen existing in solution, root growth and development is promoted, which benefits plants to take up NH_4^+–N and NO_3^-–N. If deficient, NH_4^+–N uptake is inhibited, and roots prefer

absorption of NO_3^-–N to supplement oxygen for meeting plant needs. In this case, plant growth is retarded. The importance of aeration depends on plant species: xerophyte needs higher while hydrophiles (hydrophilic plants) need lower aeration. In good aeration soil, NH_4^+–N can rapidly transform into NO_3^-–N, and thus NO_3^-–N is the dominant form for crop uptake whereby in waterlogged rice fields, nitrification is very weak, and the NO_3^-–N produced is more easily leached and denitrified; thus, NH_4^+–N is more effective for rice (Hageman, 1984; Marschner, 1986). For good aeration in water culture experiments, automatically transferring air, adding H_2O_2, and frequent changes of nutritional solution are generally adopted (Lian, 1986; Tan, 1981).

5.4.3.4. Ammonium Concentration in Solution

Ammonium concentration in media affects the ratio of NH_4^+–N to NO_3^-–N and the total N uptake by crops. As already mentioned, Tanaka et al. (1990) found that N concentration influenced NH_4^+ and NO_3^- uptake. The uptake velocity of either NH_4^+–N or NO_3^-–N increases with the concentration in solution or media but not in a linear relation (Barber, 1984a; Epstein and Hagen, 1952). Warncke and Barber (1973) adopted five total N concentrations with different ratios of NH_4^+–N to NO_3^-–N in solution (pH 5.8) to study the uptake of the two N forms by 13-day-old maize seedlings cultured in solution for five days. The results showed that the uptake amount of NH_4^+–N or NO_3^-–N increased with the increase of the ion ratio in solution. When the N concentration in solution was 303 μmol L^{-1} and the ratio of NH_4^+–N to NO_3^-–N 2.46, maize took up the largest amount of N. and the uptake ratio of the two ions shifted gradually toward 1. Zhang et al. (1990) reported that when the N concentration was 4–8 mmol L^{-1}, with the NH_4^+–N to NO_3^-–N ratio decline, spinach biomass increased. Lu (1979) considered that the optimum NO_3^-–N rate for wheat and maize in culture solution was 100 mg N L^{-1}.

Among all the factors, the toxicity caused by NH_4^+/NH_3 to plants is a particularly important reason for plant preferences to NO_3^-–N under some specific conditions.

5.5. Toxicity of NH_4^+

Plants can tolerate high levels of soil NO_3^-, and concentration of NO_3^- may increase to several percents in the plant before phytotoxicity apparently occurs (Maynard and Barker, 1971), because plants can store a large amount of NO_3^- in vacuoles of plant tissue for reduction and use at a later

date. Accumulation of large amounts of NO_3^- in plants tends to increase the demand on carbohydrates for plant metabolism, similar to the effect of NH_4^+ in a depletion of the storage carbohydrates of plants during the assimilation of NH_4^+, following NO_3^- reduction (Michael et al., 1970). The accumulation of large amounts of NO_3^- in plant tissues might upset the cation–anion balance in the normal range 1:2 or 7:10 (Nye, 1981) of plants by increasing much more anion uptake than cations and by further impacting the plant synthesizing organic acid anions such as malate and citrate (Haynes and Goh, 1978; Raven and Smith, 1976). The accumulation of NO_3^- might also upset the process of osmoregulation by changing the major osmotic components of potassium salts of organic acids and sugars (Helleburst, 1976) into potassium NO_3^-. When the reduction of NO_3^-–N is not completed, nitrite N (NO_2^-) may be easily accumulated.

In addition to plant physiological properties, an excessive application of N fertilizer is the main cause for large amounts of NO_3^-–N accumulation in plants (Lorenz, 1978; Maynard, 1978; Maynard et al., 1976; Mills and Jones, 1979; Minotti, 1978) that may be toxic to humans, particularly infants and livestock that use such plant products as food or feeds (Haynes, 1986).

Any way, although an excess supply of NO_3^- might be toxic to plants (Mills and Jones, 1979), the opportunity is much less than for NH_4^+–N. The preference of the majority of plant species to NO_3^-–N rather than to NH_4^+–N may relate to the serious toxic effects of high levels of ambient NH_4^+ or NH_3 on plant growth, and thus in reality, only a few plants can use NH_4^+–N as a sole N source (Kronzucker et al., 1996, 1997). With the supply of a large amount of NH_4^+–N as the sole N source, some crops especially vegetables in solution culture often grow poorly, and their biomass reduces, photosynthetic velocity declines, and roots retard (Cao and Li, 2003a, 2003b, 2004a, 2004b; Cao et al., 2003; Gerendás et al., 1997; Sun et al., 2004). Although low levels of NH_4^+–N have no great influence on enzyme activities in the photosynthetic process, and may increase chlorophyll contents (Raab and Terry, 1994), high levels of NH_4^+–N can produce toxicity, seriously inhibit crop plant growth, reduce leaf areas, and damage the photosynthetic structure, and disturb normal metabolism (Tan and Li, 2005; Tian et al., 2003).

5.5.1. Appearance of Toxicity

In comparison with NO_3^-–N, NH_4^+–N obviously inhibits the accumulation of plant biomass (Fan et al., 2005a; Qiu and Zhang, 2003; Zou et al., 2004). In fertile soils, the nitrification process is generally rather rapid, and therefore, the NH_4^+ or NH_3 cannot reach a level that is phototoxic to

plants, and NH_4^+ toxicity does not occur. Under a condition with nitrification being seriously inhibited, a longterm supply or heavy application of large amounts of NH_4^+–N as the sole N source followed by cool soil conditions that inhibit nitrification and make NH_4^+–N predominant in the soil can produce toxic effects to plants. Banding application of urea and anhydrous NH_3 can make NH_4^+ or NH_3 too high in a small volume and can result in the toxicity (Bennett and Adams, 1970; Court et al., 1964). Workers, using a variety of plants, have suggested many different reasons for the several toxic effects of NH_4^+ (Goyal et al., 1982a, 1982b; Haynes and Goh, 1978; Reisenauer et al., 1982). These toxic effects depend on the experimental conditions encountered and the plant species involved.

The toxicity of NH_4^+ or NH_3 is, as a whole, characterized by an immediate restriction in plant growth, stem lesions, leaf area decline, total biomass reduction, and finally death (Barker et al., 1966a, 1966b; Maynard and Barker, 1969; Maynard et al., 1966, 1968). Such phenomena have been observed in bean (Chaillou et al., 1986), sugar beet (Van Beusichem et al., 1988), tomato (Magalhaes and Huber, 1989), maize (Cramer and Lewis, 1993), oilseed or palma-Christi (*R. communis*) (Raab and Terry, 1994), and strawberry (*Fragaria viridis*) (Claussen and Lenz, 1999).

Particular symptoms such as chlorosis of leaves, marginal necrosis, necrotic spots, or simply, yellowing, and necrosis, of NH_4^+ toxicity can occur in a severe case. These symptoms are apparently associated with the disruption of the structure and integrity of chloroplasts. Puritch and Barker (1967) found a severe disruption of the chloroplasts of NH_4^+-toxic leaves and its induced impairment of photosynthetic capacities of plant tissue. Goyal et al. (1982a, 1982b) observed that the chlorophyll content of leaves of plants fed with NH_4^+ as a source of N decreased rapidly and continuously although at less than toxic levels, NH_4^+ nutrition might have a positive influence on the development of chloroplasts (Golvano et al., 1982).

Retardation or restriction of root growth and development (Gerendás et al., 1997; Raab and Terry, 1995) is a common phenomenon due to the decline of photosynthetic velocity, and carbohydrate transport from leaves to roots, and production of short, thick, less branched, and darkly colored roots is the particular feature of the toxicity (Cox and Reisenauer, 1973; Maynard and Barker, 1969). Warncke and Barber (1973) observed that root growth was restricted more than top growth so that the shoot: root ratio increased. The toxic effect also is seen physiologically on the decrease of photosynthesis velocity (Gerendás et al., 1997; Raab and Terry, 1995).

A wilting phenomenon has been observed as a symptom of NH_4^+ toxicity by several workers (Maynard and Barker, 1969; Suart and Haddock, 1968). Such a water-stress occurrence due to NH_4^+ nutrition has appeared with the decrease of plant water uptake, xylem exudation, and leaf water potential (Pill and Lambeth, 1977; Pill et al., 1978; Quebedeaux and Qzbun, 1973). Pill and Lambeth (1980) associated blossom-end rot in tomatoes under NH_4^+ nutrition with a reduced transpiration rate resulting in reduced water flux into fruit.

Different species may have different tolerances to the NH_4^+ or NH_3 (Haynes and Goh, 1978), and thus, the serious magnitude of the toxicity can appear to be different among species and even among cultivars in the same species. Lasa et al. (2001) postulated that the sensitivity of plants to NH_4^+–N was related to N distribution, and the size for NH_4^+–N assimilation was a determined factor. Accumulation of NH_4^+–N in plant stems can lead to the reduction of biomass while assimilated in roots, the plants will have more tolerance to NH_4^+–N.

5.5.2. Mechanisms of the Toxicity

Obviously, the toxicity of NH_4^+ to plants is the combinative result of internal and external factors. Depending on the plant species and particular growth conditions, each of these factors may contribute to plant NH_4^+ toxicity.

Various hypotheses have been put forward to explain the physiological process of NH_4^+ toxicity. It has been found from the whole tissue analysis that the NH_4^+-fed plants accumulate more NH_4^+ ions, inorganic anions (chloride, sulfate, and phosphate), and amino acids while reducing the concentration of the essential cations (K^+, Ca^{2+}, and Mg^{2+}) and organic acids such as malate compared to NO_3^--fed plants (Britto and Kronzucker, 2002). Such observations have led to the hypotheses that NH_4^+ toxicity may be the result of (1) decreases in the uptake of essential cations (Roosta and Schjoerring, 2007; Siddiqi et al., 2002); (2) NH_4^+-induced disorders in pH regulation; (3) excessive consumption of sugars for NH_4^+ assimilation causing carbohydrate limitation (Finnemann and Schjoerring, 1999). The NH_4^+-sensitive species barley having a much higher influx of NH_4^+ into the roots than the NH_4^+-tolerant species rice suggests that the former has a high ability in controlling the influx of NH_4^+ into the roots (Britto et al., 2001). In contrast, barley releases NH_4^+ back into the soil. This has led to such a hypothesis that in NH_4^+-sensitive species, the apparently futile transmembrane cycling of NH_4^+ and the operation of an energy-intensive NH_4^+ efflux mechanism may be the cause of NH_4^+ toxicity (Britto et al.,

2001). Additionally, the acidification of the rhizosphere induced by NH_4^+ uptake may in itself pose a stress in plants, particularly in acid soils where it can increase Al toxicity.

Presently, there exist two views on the toxicity source: NH_3 toxicity and NH_4^+ toxicity and the mechanism for neither is fully understood.

5.5.2.1. NH_3 Toxicity

It has been found that NH_4^+–N in solution can be toxic to plants. The toxicity is believed to be caused mainly by free NH_3 that affects plant growth and metabolism at low concentration levels at which NH_4^+ is found to be not harmful. It is well known that the distribution of NH_4^+ and NH_3 in aqueous solution maintains equilibrium, and the NH_3 concentration depends very much on the pH of the media. At high pH, NH_4^+ shifts over to NH_3 that can cause toxicity. A typical example of NH_3 toxicity has been demonstrated by Bennett (1974). The NH_3 (aq) particularly affects root growth even at very low concentrations of $0.2\,mol\,m^{-3}$ (Bennett and Adams, 1970) and impairs seed germination (Barker et al., 1970). The presence of NH_4^+/HN_3 in a nutrition solution leads to an immediate increase in the cytosolic pH because of the uncontrolled NH_3 penetration to the plasma membrane and the vacuole. This high cytosolic pH inhibited GS in maize but did not affect GS activity in rice since the cytoplastic pH in rice was regulated downward (Kosegarten et al., 1997). These results show that the inhibition of the GS by high cytosolic pH is the primary cause of NH_3 toxicity. Such a reaction for the inhibition of GS indicates that the whole N metabolism is blocked with disastrous consequences for growth. Gerendás et al. (1990) proved that in the presence of NH_4^+/HN_3, a high pH in the nutrient solution had increased the pH in both the cytosol and vacuoles also due to the uncontrolled penetration of HN_3 into these compartments. Such findings are in agreement with the results of Kosegarten et al. (1997).

Wilson et al. (1998) found that even at a low pH of 5.0 where no NH_3 is present in the nutrient solution, the vacuolar pH in maize roots was increased to a relatively high level and could not be restored, whereas in rice roots at the same external pH 5, there was only a minor vacuole pH increase. Because of the high pH in the cytosol, some NH_4^+ is deprotonated and HN_3 may penetrate the tonoplast and increase the vacuole pH. This result not only shows that plant species may differ in their sensitivity to NH_3 toxicity but also that at low pH in the nutrient solution at which no NH_3 is present, NH_3 toxicity may occur if the NH_4^+ concentration of the

nutrient solution is high to 2 mol m^{-3}. A high vacuole pH implies a decrease of the proton gradient across the tonoplast with a consequent depression in anion accumulation in the vacuole and other metabolic processes. From these results, it is considered that the toxicity is mainly caused by free NH_3 (Cramer and Lewis, 1993). The initially toxic concentration of water soluble NH_3 is 0.15 mmol L^{-1}, and the lethal concentration is 6.0 mmol L^{-1} (Bennett, 1974). The NH_3 toxicity is affected by pH and NH_4^+ concentration. A higher pH benefits NH_3 (in water) formation and produces NH_3 (in water) toxicity easily. Under acid conditions, H^+ concentration is high, and can decrease NH_3 (in water) formation, increases the NH_4^+ concentration, and thus crops can tolerate higher NH_4^+.

Rice is regarded as a typical crop that prefers NH_4^+–N, and many workers have focused on studying NH_3 toxicity to rice. The magnitude of NH_3 toxicity to rice is considered in relation with cultivar characteristics, substances contained in cells, changes of root pH environment, and their induced soil plant nutrition obstacles. In general, the NH_4^+ that enters cells has no toxic effect on plant metabolism process (Mengel and Kirkby, 2001) and the toxicity appears only when it is accumulated in large amounts (Haynes and Goh, 1978) and a certain amount of NH_3 is liberated. In soil with alkaline properties, $NH_4^+$is able to transform into NH_3 (Mengel, 1991) and with the increase in pH, the NH_3 ratio and its toxicity continuously increase (Mengel and Kirkby, 2001). Different from NH_4^+, NH_3 molecules can directly penetrate the membrane and enter cells through diffusion (Wilson et al., 1998), whereas other cations existing in solution have no possibility of inhibiting NH_3 absorption by antagonism through competition of ion channels (Feng et al., 1999). It is believed that the decline of GS activity is the primary cause of NH_3 toxicity to rice (Gerendas and Ratcliffe, 2000; Howitt and Udvardi, 2000) as reported by Wilson et al. (1998). The difference in proton pump activity of various plant species controls plant sensitivity to NH_3 toxicity (Kosegarten et al., 1999). Rice can actively regulate cytoplasm pH under alkaline conditions, and make GS activity to be unaffected, and therefore prevents NH_3 toxicity (Wilson et al., 1998).

5.5.2.2. NH_4^+ Toxicity

Although NH_4^+ toxicity has been proposed for a long time, direct evidence is still lacking. Recently, a very interesting phenomenon has been observed by some researchers. Under culture conditions with water stress, various cultivars of rice show a relative preference to NO_3^-–N, while the NH_4^+ treatment makes rice growth to be retarded. However, under solution culture

without water stress, they relatively prefer NH_4^+–N. Soil pot experiments with different ratios of NH_4^+–N to NO_3^-–N using same water management obtained very similar results (Qian et al., 2003). Application of NH_4^+–N alone has produced NH_4^+ salt toxicity, and it occurs more easily under water culture conditions. When the pH is <7.25, 99% of the NH_3 is changed into NH_4^+ by protonation, and therefore, such toxicity cannot be attributed to NH_3 that has been considered to have a relatively high toxicity to plants, The concentrations of NH_4^+ in the cytosol range from 1 to 30 mM (Miller et al., 2001). Excessive accumulation of NH_4^+ in the cytosol may lead to necrosis of plant tissue. Determination shows that the pH in the plant-cultured solution containing NH_4^+–N is only higher than 5 during the culturing process, and there is no opportunity for the pH to be high enough to form large amounts of NH_3 molecules that can enter cells by diffusion, and when rice is stressed by water, the absorbed NH_4^+ cations are significantly reduced, and there is no excessive accumulation of NH_4^+ cations in plants (Qian et al., 2003). Rice possesses relatively independent systems for the transport of NH_4^+–N and NO_3^-–N (Glass et al., 2001), and thereby, such a phenomenon is not a single competition. This shows the preference of NH_4^+–N being condition dependent: under a sufficient supply of water, it has been proven much earlier that the existence of NH_4^+–N can reduce the NO_3^- uptake for some plants, but the existence of NO_3^-–N does not affect NH_4^+ uptake (von Wiren et al., 1997). In some experiments, either the condition for producing the toxicity of NH_3 or the effect induced by pH changes is eliminated, the effect of NH_4^+on inhibition of plant growth still exists under the simulated-water-stress condition. Based on this fact, many scientists believe that the toxicity is caused by NH_4^+, at least to some extent, and not by NH_3, and the toxicity mechanism is related to NH_4^+-induced or intensified damage. Injury of membrane and cell wall structure as well as membrane enzyme activity is regarded as the primary cause. Following this, the retardation of nutrient absorption, changes of enzyme activities, and metabolism will be induced and plant growth be hindered as the secondary cause. Such a view of NH_4^+ toxicity is supported by some indirect evidences.

First, during the N absorption, assimilation and transport processes, the transport of N across the membrane may be first affected by NH_4^+. Research shows that the NH_4^+ absorption is implemented by NH_4^+ carriers located in membranes and that some genes in relation to the process have been coded and studied (Howitt and Udvardi, 2000). Water stress has an unfavorable effect on membrane stability and NH_4^+–N may have a high probability to intensify the ill effect.

Second, the existence of a large amount of NH_4^+ under a water-stressed condition may make ATPase in membranes difficult to display its role in pumping H^+ as under normal conditions, decline membrane potential, and result in cell H^+ accumulation and changes of some related enzyme activities. In this case, reduction of the net uptake of NH_4^+ due to the decline of pH accompanies an obvious decrease in the mole ratio of the net exudative H^+ to the net uptake of NH_4^+ (Dyhr-Jensenl and Brix, 1996). H^+ exudation from inside the membrane to outside the membrane for maintaining a certain membrane potential depends mainly on the function of H^+-ATPase in the membrane (Kurdjian and Guern, 1989), while the NH_4^+ uptake can lead to the decline of membrane potential, and thereby makes the membrane depolarized (Wang et al., 1993, 1994).

Third, the existence of NH_4^+ may induce or promote the dissociation of Ca^{2+} in the membrane under water-stress conditions, and make the membrane function difficult to maintain. It has been proposed that in many polarized areas of a membrane, Ca^{2+} maintains the membrane stability by forming a calcium bridge with the membrane, and thus, it cannot be absent for the formation of new membrane (Minorsky, 1985). Caldwell and Haug (1982) reported that Ca^{2+} was mainly adsorbed in the negatively charged phosphate group of the membrane ester (phospholipids), and in this way limited soluble solutes from penetrating the membrane. Once the Ca^{2+} was removed from these sites, the membrane would be broken and decomposed, and some important substances would penetrate out of cells and cause damages (McLauglin and Wimmer, 1999). Several researchers in the past have focused on the membrane damages due to the substitution of H^+ for Ca^{2+}, but whether NH_4^+ participated in the substitution of H^+ for Ca^{2+} remains unknown. Some results indicate that under a simulated-water-stress condition, the Ca^{2+} concentration in the nutritional solution after culturing rice for a period of time did not decrease, but instead increased. Although it is impossible to know exactly how much Ca^{2+} comes from membrane dissociation, according to the investigation by Murtadha et al. (1988), the higher NH_4^+ concentration in the external environment can make the release of Ca^{2+} adsorbed in anionic sites of the cell wall. Thereby, under water-stress conditions, it is possible that NH_4^+ participates in the substitution process for Ca^{2+} in membranes and therefore accelerates the membrane destructive process.

Fourth, under the water-stressed conditions, NH_4^+ can induce or strengthen some ion leakages out of roots, and bring about nutrient loss. Under a water-stress condition that is simulated, the supply of NH_4^+ as the sole N source caused NO_3^- leakage out of the roots of rice plants and

reduced the absorbed amounts of many nutritional ions (Qian et al., 2003). The major reasons may include (1) ATPase activity in the membrane is inhibited, and thus leads to NO_3^- leakage out of roots; (2) uptake of NH_4^+ may bring about the decline of the membrane potential and strong depolarization of the membrane, and this does not benefit a variety of ion absorption; and (3) decrease in membrane stability and the selectivity of nutrient absorption result in ions such as NO_3^- leaking out of roots.

Last, under water-stress conditions, NO_3^- nutrition may have a significant role in alleviating the NH_4^+ toxicity to rice. A number of experiments show that partial substitution of NO_3^- for NH_4^+ has obviously improved plant growth. Even though under a simulated-water-stress condition the NH_4^+ uptake significantly decreased, the NO_3^- uptake increased.

5.5.3. *Factors Affecting Toxicity*

NH_4^+–N may be toxic to plants in two ways: by a direct effect of NH_4^+ or NH_3 or by an indirect effect by influencing environmental conditions and then influencing crop plants.

For the indirect effect, NH_4^+–N can result in a series of effects on environments, and these effects will in turn bring about toxicity to plants.

5.5 3.1. Cation Deficiencies

Cation deficiencies caused by NH_4^+–N nutrition have been regarded as a reason for the preference of plants to NO_3^-–N. The uptake of NH_4^+–N and NO_3^-–N by crops affects their uptake of other cations and anions: NH_4^+–N uptake can promote anion uptake, while NO_3^-–N uptake can promote cation uptake and inhibit anion uptake (Haynes, 1986; Kirkby, 1968) although by using NH_4^+ as the N source, the cation uptake by crops is dominant while by using NO_3^- as the N source, anions are the dominant ions by crop uptake (Barber, 1984a, 1984b). In association with NH_4^+ toxicity, the NH_4^+ nutrition can inhibit the uptake of some cations such as Ca^{2+}, K^+, and Mg^{2+} by plants (Gerendás et al., 1997). This has been observed in the decline of fruit Ca concentration (Pill et al., 1978), deficiencies of K and Ca occurring quite often in plants (Hoff et al., 1974; Maynard et al., 1968) and appearance of tomato blossom-end rot (a physiological disease associated with Ca deficiency) in comparison with that of NO_3^- (Pill and Lambeth, 1980; Pill et al., 1978; Willcox et al., 1973). Such phenomena have been attributable to ionic antagonism or competition (Black et al., 1969) during cation uptake either with NH_4^+ ions per se or with H+ ions excreted during active NH_4^+ uptake. The fact that adding

excessive amounts of ambient K has corrected the K deficient symptoms caused by NH_4^+ toxicity provided evidence to support the assumption (Ajayi et al., 1970). In contrast, Cao et al. (2003) reported that NO_3^- had promoted K^+ absorption, and Zou et al. (1996) revealed that NO_3^- had increased calcium and magnesium absorption.

Calcium ion plays a certain role in the promotion of plant growth and in plant responses and adaptation to environments. As a secondary message of cells, calcium has a great function in the stabilization and maintenance of plant cell wall, cell membrane, and the penetration of cell membranes, regulation of plant enzymes, and balance of anions and cations, and thus influences other nutrient uptake and transport in plants. Promotion of cation absorption at low pH by Ca^{2+} may be caused by its maintenance of the integrity of the cell membrane. As an example, external addition of calcium to maize promoted root growth and development as well as biomass increase. Tang et al. (1999) proved that at low pH, activation of calmodulin needed more Ca^{2+}, and calcium played, to a certain extent, an active role in resistance to low pH and high NH_4^+ concentration toxicity in the inhibition of plant growth. It is now considered that high H^+ can replace the divalent or trivalent ions in the membrane and destroy the membrane, leading the membrane to lose its ability in controlling penetration (Liao and Yan, 2003). This may be the reason why high H^+ concentration can lead to the retardation of cation absorption and significantly influences root development.

Not only does the NH_4^+ and NO_3^- uptake affect other cation and anion uptake but it also affects the type and contents of anions and cations in the nutritional media affect crop uptake of NH_4^+ and NO_3^-–N to various extents (Barker et al., 1974a, 1974b; Beevers, 1976; Epstein, 1972, 1976; Kirkby, 1968; Loo, 1931; Ni and An, 1984; Schjorring, 1986). Other studies show that Ca^{2+}, Mg^{2+}, K^+, and SO_4^{2-} can promote NO_3^-–N uptake while inhibiting NH_4^+–N uptake (Haynes, 1986). Schjorring (1986) reveals that the existence of P in media can promote barley, buckwheat, and rape to take up more NO_3^- and NH_4^+–N than in the absence of P.

NH_4^+ and NO_3^- have a certain interaction in affecting each uptake. When NH_4^+–N and NO_3^-–N coexist in the media, NH_4^+–N can inhibit some crops, such as rice and wheat, to absorb NO_3^-–N, while NO_3^-–N has no such effect on NH_4^+–N uptake (Beevers, 1976; Fried et al., 1965; Mengel, 1983; Minotti et al., 1969a, 1969b; Munns and Jackson, 1978; Youngdahl et al., 1982). Experiments from Lycklama (1963) show that with the increase of NO_3^-–N in solution, the uptake of NH_4^+–N slightly decreased.

Such a result was proved by a ^{15}N-labeled experiment of rice conducted by Mengel et al. (1976). However, some other results show that NH_4^+–N does not affect NO_3^-–N uptake (Edwards and Barber, 1976; Schrader et al., 1972) or the effect is small as shown in China squash (*Cucurbita*) or musky pumpkin. For the mechanism of NH_4^+–N inhibition of NO_3^-–N, some scientists hold the view that NH_4^+–N inhibits the NRA and thereby affects NO_3^-–N assimilation (Mengel and Kirkby, 1987), while others consider that NH_4^+–N affects the process of NO_3^-–N uptake, but not its reduction (Minotti et al., 1969b).

5.5.3.2. The pH Changes

The uptake of different ions by plants may produce different effects on media pH, and that of the two forms of N sources has exerted a great effect on rhizosphere pH. The plant toxicity induced by NH_4^+ is related to strong acidification of rhizospheric soils and out control of the cell pH (Bligny et al., 1997; Gerendás et al., 1997; Ishiyama et al., 2004a, 2004b; Kronzucker et al., 1997; Yamaya and Oak, 2004). Allen and Raven (1986) report that when plants absorb NH_4^+, equal amounts of H^+ will be released outside roots to balance the charges of plants, and this will induce the decrease of pH in the rhizosphere. This may be caused by the excessive uptake of cations over anions, and therefore, roots must secrete H^+ to maintain the balance between cations and anions within plants (Marschner, 1995). Due to the uptake of NH_4^+, the cell membrane may be depolarized, and a large amount of protons will be secreted (Schumaker and Sze, 1990; Wieder et al., 1990). Under acidic conditions, plants may have an acid-tolerant ability (Brix et al., 2002). For these reasons, when plants are nourished with NH_4^+, acidification of the plant rhizosphere occurs, and the medium pH declines. In contrast, when NO_3^-–N is taken up by plants, the medium pH increases. This common phenomenon can be easily observed in solution culture rather than in fields. Due to the difference in soil type and crop species, the pH changes in rhizospheres must be different (Wu and Liu, 1985). Many workers have demonstrated that the NH_4^+ uptake by plants accompanies the decline of medium pH, while NO_3^- uptake increases the medium pH (Barber, 1984a; Fried et al., 1965; Jungk, 1970; Van den Honert and Hooymans, 1955). The uptake of different N forms is linked to H^+ production and charge balance. In aerobic soils, the transformation of NH_4^+–N into NO_3^-–N by nitrification also leads to pH changes (Hageman, 1984). During the nitrification process, oxidation of 1 mol of NH_4^+–N releases 2 mol of H^+ ions and thus leads to a decrease in soil pH. This is the accepted

reason for accelerating the acidification of soil by the application of NH_4^+–N fertilizers (Barak et al., 1997; Guo et al., 2010; Lungu and Dynoodt, 2008; Malhi et al., 1998; Schroder et al., 2011; Wen et al., 2011; Xu et al., 2002b; Zeng et al., 2005; Zhao et al., 2007).

The significant changes of rhizosphere pH have in turn affected crop uptake of the two N forms (Barber, 1984a; Hageman, 1984; Mengel, 1983), and the relation of pH to the uptake of NH_4^+–N and NO_3^-–N is interacted or interrestricted. Mengel and Kirkby (1987) pointed out that the difference in the NH_4^+–N and NO_3^-–N uptake by different crops was mainly caused by their sensitivity to pH. Schubert and Yan (1997) studied the effect of NH_4^+ and NO_3^- on pH changes inside root cells and outside root cells and H^+-ATPase activity in plasma membranes. Their results illustrated that NH_4^+ and NO_3^- absorption had greatly altered the cell pH and the H-ATPase activity in relation to various physiologies. As a rule, low pH in media benefits NO_3^-–N uptake, whereas high pH increases NH_4^+–N uptake. Experiments from Prianishnikov (1945) showed that in neutral reaction (pH 7), the effect of NH_4^+–N was superior to that of NO_3^-–N, while in an acid condition (pH 5), the reverse was true. Ni (1980a, 1980b) reported that when pH increased from 6.0 to 7.4, the NO_3^-–N uptake by wheat was reduced by 50%. Vessey et al. (1990) studied soybean responses to various ratios of NH_4^+–N to NO_3^-–N by water culture under various pH conditions and found that the uptake ratio of NH_4^+–N to NO_3^-–N was decreased with the decrease of pH, and when the pH was 6.0, the ratio of the two N sources was 2:1. Michael et al. (1970) studied some crop responses to NH_4^+–N and NO_3^-–N and revealed that at pH 5.8, tomatoes had an equal velocity for the uptake of either of them; when the pH increased from 4.0 to 7.6, the absorbed NH_4^+–N amount was increased by almost 1-fold, while NO_3^-–N was decreased by 38%. At pH 6.8, barley took up almost the same amount of NH_4^+–N and NO_3^-–N.

The pH in soil solution and the uptake of different N forms affect the pH in root cells (Mengel and Geurtzen, 1986), and thereby the membrane stability and the penetration of selectively absorbed nutrients. Once taken up by plants, NH_4^+–N is assimilated rapidly into amino acids in root cells and is then transferred in the amino acid form to shoots. This may be the reason why NH_4^+ is seldom detected in the xylem vessels. Lasa et al. (2001) postulated that the site for the assimilation of NH_4^+–N was a key factor in determining plant tolerance to NH_4^+. When assimilated in roots, plants will tolerateNH_4^+–N, while when accumulated in upper parts, the plant biomass will be decreased. Therefore, the NH_4^+–N concentration in the

xylem is closely associated with plant dry matter production. The increase in the pH in the xylem sap has been believed to be a regulative signal under soil dry and unusual external pH conditions by some workers (Gollan et al., 1992; Wilkinson and Davies, 1997). However, Wilkinson et al. (1998) consider that the pH increase is not a signal but a phenomenon of injury under both unusual external pH and water-stressed conditions. Soil dryness, as a stress, can lead to plant injury that may induce some mechanisms to regulate the sap pH of the xylem, so such a change of the pH in the xylem is a passive process. Liu et al., (2004) revealed that dryness could increase the pH in the stem xylem, and there was no correlation between the xylem pH and the external solution pH. The unusual pH value is a type of environmental stress to plants, especially to plant lateral roots, whereas large amounts of NO_3^-–N can promote the occurrence of lateral roots (Cramer and Lewis, 1993; He et al., 1999; Sattelmacher and Thoms, 1995; Wang et al., 2002a).

Media acidification associated with NH_4^+ absorption has been shown to be toxic to many crop plants such as peas, beans, maize, tomatoes, and asparagus (*Asparagus officinalis*) (Maynard and Barker, 1969; Maynard et al., 1966) in another way. Claussen and Lenz (1995) revealed that in maintaining the balance of cations and anions in the plant body, release of H^+ to the rhizosphere in the process of absorption of NH_4^+–N by plants made the rhizosphere acidified, and root growth inhibited An early maize experiment showed that with NH_4^+–N nutrition, the yield was significantly decreased when the pH was either lower or higher than the most suitable pH of 6.0. Toxicity due to low pH values can often be alleviated by using $CaCO_3$ as a buffer to control media pH at or near neutrality (Abichandani and Patnaik, 1961; Barker and Maynard, 1972; Barker et al., 1966a, 1966b; Cao, 1965; Debrath and Mandal, 1983; He and Liao, 1989; Precheur and Maynard, 1983; Xu et al., 2002b; Zhao et al., 2007). The benefits for acidity control seem to result in greater incorporation of absorbed NH_4^+ into amino acids, amides, and ethanol-soluble N by the root tissue (Barker et al., 1966a) and therefore limits NH_4^+ transport to the shoots, where it is more toxic than in the roots. Although the reason why control of the pH of the rooting media encourages assimilation of NH_4^+ is unknown, an important phenomenon has been observed in that once NH_4^+ ions reach the shoots, the biochemistry and physiology of the plants are greatly disrupted. In contrast, NO_3^- nutrition can lead to an increase in rhizosphere pH, and therefore, the ambient concentration of NH_4^+ in excess of those required to induce toxicity symptoms can be eliminated by applying part of NO_3^- to the total ambient N. Goyal et al. (1982a) found that NO_3^- equivalent to ≥10% of

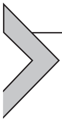
the NH_4^+ concentration alleviated the inhibitory effects of NH_4^+ on the growth of radish.

The most suitable pH for crop growth is not in agreement with the most suitable pH for crop absorption of either NH_4^+–N or NO_3^-–N (He and Meng, 1987), and the suitable pH for NH_4^+ nutrition is not that for NO_3^-–N nutrition. A solution-moving culture experiment shows that the most suitable pH for tomatoes for the uptake of NH_4^+–N is 7.0, while that for NO_3^-–N is 5.5. Hewitt and Smith (1975) considered that for NO_3^- nutrition, the most suitable pH of media was 4.5–6.0, whereas for NH_4^+ nutrition, it was 6.0–7.0. Rao and Rains (1976) and Sheat et al. (1959) reported that the pH for the maximum NH_4^+–N uptake in crops was 7–8, whereas that for NO_3^- uptake it was 4–5. However, Lycklama (1963) evidenced that the pH for maximum NO_3^-–N uptake of ryegrass was 6.2. The influence of pH is related to the N concentration in solution: when NO_3^-–N was lower ($2\,mmol\,L^{-1}$), the pH on N uptake had almost no influence; when it reached $8\,mmol\,L^{-1}$, the uptake increased with the increase in pH (Hageman, 1980, 1984). The majority of the results were obtained from water or sand culture, and the pH was the initial value of the solution; under the automatically controlled conditions, the situation may be totally different (He and Meng, 1987).

5.5.3.3. Carbohydrate Amount

The carbohydrate amount in plants substantially influences NH_4^+ toxicity. Yield reduction due to excessive supply of NH_4^+ has been, to some extent, attributed to metabolic disturbances associated with the detoxification of NH_4^+ within the roots (Reisenauer et al., 1982). Such detoxification results in an immediate demand in plants for carbon skeletons that are supplied mainly by intermediates in glycolysis and the TCA cycle (Givan, 1979). Supply of α-ketoglutarate to higher plant tissues can greatly reduce internal NH_4^+ concentration and thus alleviate NH_4^+ toxicity (Matsumoto et al., 1971). Subjected to high levels of exogenous NH_4^+, plants markedly decrease the intercellular levels of tricarboxylic acids (malic or oxalic acids), while they immediately and sharply increase the levels of cellular amides (Gln) (Kirkby, 1968; Michael et al., 1970; Reisenauer, 1978), suggesting that plants need a very high level of carbohydrates and carbohydrate-degrading reactions that provide the necessary substances for amide formation during periods of NH_4^+ assimilation. As a cellular response to the detoxification

of NH_4^+, the NH_4^+-fed plants can at least maintain and even increase their respiration rates (Berner, 1971; Goyal et al., 1982b; Ikeda et al., 1974; Wakiuchi et al., 1971; Willis and Yemm, 1955) so that they can rapidly turn over the carbon skeletons needed for NH_4^+ assimilation (Givan, 1979). Since a major effect of NH_4^+ accumulation in leaves is believed to inhibit photosynthesis and thus the production of carbohydrates (Goyal et al., 1982b) through uncoupling of noncyclic photophospholation in isolated chloroplasts (Gibbs and Calo, 1959; Krogman et al., 1959; Losada and Arnon, 1963; Ohmura, 1958), the increased respiration rate and high demand on storage carbohydrates during the growth of plants supplied with high levels of exogenous NH_4^+ are likely to be particularly damaging when the plant begins to translocate NH_4^+ to the shoots.



6. NH_4^+–N AND NO_3^-–N NUTRITION AT DIFFERENT STAGES OF PLANTS

6.1. Crop Responses to NH_4^+–N and NO_3^-–N at Different Stages

Researchers have been paying much attention to the fertilization of NH_4^+–N and NO_3^-–N in field experiments and have used the kinetic parameters obtained from crop seedlings to explain the preferences of different crops or genotypes to NH_4^+–N or NO_3^-–N. However, whether the preferred degree and the preferred ratio of NH_4^+–N to NO_3^-–N of crops at different stages are in agreement with the seedling stage remains unknown.

Soil as a plant-growing medium can influence the absorption in multiple ways. For this reason, field experiments and pot experiments using soil as cultural media are unsuitable for such studies. Water culture conditions are easy to control, and NH_4^+–N and NO_3^-–N in solution do not change except by crop uptake, and are thus at a constant level.

He et al. (1999) adopted solution culture to study the characteristics and amounts of NH_4^+–N and NO_3^-–N absorption of six crops during the entire growth period in addition to their kinetic parameters at the seedling stage. In implementation of the experiments, they designed a control solution in which plants were not cultured. The control was treated in the same way as other treatments such as alteration of the solution and analysis of NH_4^+–N and NO_3^-–N at an interval of time. Results show that in any case, no matter how long the solution was altered, and no matter whether the culture season was in spring or in summer, the NH_4^+–N and NO_3^-–N amounts

Table 5.8 Concentrations (mg N L^{-1}) of NH_4^+–N and NO_3^-–N in Solutions with Different pHs

Date/month	pH 5.0		pH 6.5		pH 8.0	
	NH_4^+–N	NO_3^-–N	NH_4^+–N	NO_3^-–N	NH_4^+–N	NO_3^-–N
			Controlled solution			
16–26/3						
2/4	42.19	42.08	41.50	42.00	40.60	42.16
9/4	41.10	42.84	40.86	42.00	40.00	42.05
23/4	42.90	42.43	42.15	42.89	42.00	42.52
7/5	41.16	42.24	41.30	42.00	41.20	42.00
28/5	41.35	41.00	41.72	41.46	40.61	43.00
11/6	42.40	42.24	42.00	42.43	42.68	42.40
21/6	41.58	42.43	41.60	42.15	41.13	42.90
11/7	41.88	43.30	41.50	42.90	41.11	42.70
CV (%)	1.53	1.56	0.97	1.16	2.05	0.90
			Wheat-cultured solution			
16–26/3						
2/4	41.66	40.13	40.25	40.48	38.92	41.49
9/4	39.45	36.90	37.50	37.33	35.62	40.29
23/4	37.51	29.91	33.13	31.92	32.09	38.32
7/5	30.93	23.96	27.07	24.99	25.90	31.39
28/5	21.24	8.71	12.36	10.36	6.55	24.76
11/6	31.63	27.74	29.34	28.25	25.89	34.08
21/6	35.22	34.20	33.06	34.84	33.58	38.68
11/7	35.98	36.50	29.30	32.60	34.11	39.20

Note: initial concentration was 42.00 N mg^{-1} L^{-1} for either NH_4^+–N or NO_3^-–N. Solution volume was 1000 ml per pot, and two plants of wheat were cultured in each pot.
He et al. (1999).

and their ratio had no obvious changes. In three solutions with different pH values, the concentrations of NH_4^+–N and NO_3^-–N in controlled solutions were almost the same as the initial concentration (42.00 mg L^{-1} N) after a certain time. The averaged change in the amounts of NH_4^+–N was ±0.68 mg L^{-1} N, and that of NO_3^-–N was ±0.51 mg L^{-1} N, and the relative error expressed as a percentage of the initial concentration was 1.52% for NH_4^+–N and 1.21% for NO_3^-–N, both being in the range of allowable values of determination by the colorimetric method. In contrast, the concentration changes for plant-cultured solutions were several times larger than that of the controlled solution. Therefore, consideration of the controlled solution change or not does not affect the results. For strict reasons, the changed amount of NH_4^+–N and NO_3^-–N in the controlled solution was subtracted from the plant-cultured solution (Table 5.8).

During the culture period, the concentration of NH_4^+–N and NO_3^-–N was determined at each time there were solution changes. The difference between the initial concentration and that changed with time was calculated, and the reduction concentration times the volume is regarded as the plant uptake N of either NH_4^+ or NO_3^-, and the total cumulative uptake (mg N plant^{-1}) was the sum of the determined amount of each stage. The ratio of NH_4^+–N to NO_3^-–N in the period of culture by plant uptake was called the relative uptake amount or the uptake ratio, and the ratio calculated from the cumulative amount was called the averaged ratio. When the ratio was <1, or the absorbed NH_4^+–N accounted for <50% of the total N absorbed, these crops were regarded as NO_3^-–N-preferring crops; otherwise, they were regarded as NH_4^+-preferring crops (Table 5.9).

Results show that crops grown in solution with equal amounts of NH_4^+–N and NO_3^-–N did not absorb equal amounts of each N form, varying not only with crop species, but also regularly with growing stages of each crop. Grown in solution at an initial pH of 6.5, the wheat uptake ratio of NH_4^+–N to NO_3^-–N was increased with growing duration, ranging between 0.67 and 1.18. At the tillering stage (grown for about 38 days), from elongation to heading stages (grown for 49 days) and from flowering to maturing stages (grown for 31 days), the ratios were 0.79, 0.90, and 1.18, respectively. The NH_4^+–N uptake in the three stages were 44.2%, 47.5%, and 54% of the total N, respectively. In such a solution, wheat preferred NO_3^-–N at early stages, whereas NH_4^+–N at late stages. As a whole, the ratio absorbed was 0.94, and the cumulative NH_4^+–N absorbed was 48.3% of the total.

The uptake ratio of maize varied between 0.4 and l.42. The ratio at the seedling stage (grown for 51 days), spike stage (from the51st to 72nd days), and flowering and seed formation stage (from the 72nd to 114th days) was 1.32, 1.03, and 0.61, respectively, and the proportion of NH_4^+–N absorbed was 57%, 50.7%, and 38% of the total N at each stage, respectively. Obviously, maize took up more NH_4^+–N at the seedling stage, almost the same amount of either at the spike stage and more NO_3^-–N at the flowering and seed formation stage. In the entire period, the two forms' N ratio was 0.74, cumulative NH_4^+–N was 42.7% of the total N, and thus belongs to an NO_3^-–N-preferring crop.

The absorbed ratio for proso varied from 0.80 to 1.00 with a relatively small range, and it had a decreasing trend with the progress of plant growth: the averaged uptake ratio was 0.96, 0.90, and 0.87, respectively, for the seedling stage (before day 49), from elongation to the heading stage (from day 49 to day 77), and from flowering to maturing (from day 77 to day 110),

Table 5.9 Amounts and Ratios of NH_4^+–N and NO_3^-–N Absorbed by Crops at Different Growth Stages

Days of growth	Cumulative N absorbed (N mg plant^{-1})			Ratio	Days of growth	Cumulative N absorbed (N mg plant^{-1})			Ratio
	NH_4^+	NO_3^-	NH_4^++NO_3^-	NH_4^+/NO_3^-		NH_4^+	NO_3^-	NH_4^++NO_3^-	NH_4^+/NO_3^-
		Wheat					**Maize**		
10					9				
17	0.62	0.76	1.34	0.82	23	7.45	7.55	15.00	0.99
24	1.68	2.34	4.02	0.67	37	23.47	18.85	42.32	1.42
38	6.19	7.82	14.01	0.82	51	71.79	54.24	126.03	1.36
52	13.30	16.32	29.62	0.84	58	141.29	118.61	259.90	1.08
73	17.98	31.88	59.86	0.94	65	220.60	191.28	411.88	1.09
87	34.32	38.96	73.28	0.89	72	353.40	328.10	681.48	0.97
97	38.58	42.62	81.20	1.17	86	633.70	753.30	1387.00	0.66
117	44.68	47.77	92.45	1.18	100	791.90	951.10	1743.00	0.80
					114	863.30	1159.20	2022.50	0.34
		Proso					**Tomato**		
7					14				
21	2.64	2.81	5.36	0.94	28	2.50	2.26	4.76	1.11
35	12.58	12.71	25.29	1.00	42	21.65	17.65	39.30	1.24
49	31.94	33.29	65.23	0.94	56	57.28	52.42	109.72	1.02
56	59.40	67.80	127.20	0.80	63	113.19	123.06	236.25	0.79
63	89.86	99.79	189.65	0.95	77	172.43	188.65	361.08	0.90
70	127.00	140.82	267.82	0.90	91	217.83	234.85	452.68	0.98
77	166.45	181.76	348.21	0.96	105	275.07	296.75	571.82	0.92
84	205.56	223.63	429.19	0.93	119	336.50	366.81	703.30	0.88
98	266.45	294.76	561.21	0.86	133	398.48	436.58	835.06	0.89
110	292.74	327.21	619.95	0.81	147	453.69	500.94	954.63	0.86

Continued

Table 5.9 Amounts and Ratios of NH_4^+–N and NO_3^-–N Absorbed by Crops at Different Growth Stages—cont'd

Days of growth	Cumulative N absorbed (N mg plant^{-1})			Ratio	Days of growth	Cumulative N absorbed (N mg plant^{-1})			Ratio
	NH_4^+	NO_3^-	NH_4^++NO_3^-	NH_4^+/NO_3^-		NH_4^+	NO_3^-	NH_4^++NO_3^-	NH_4^+/NO_3^-
		Pakchoi					**Buckwheat**		
14					14				
28	0.81	1.10	1.91	0.74	21	2.43	2.78	5.21	0.87
42	6.18	8.35	14.53	0.74	28	4.14	4.60	8.74	0.94
49	11.08	13.61	24.69	0.93	35	6.20	6.24	12.44	1.26
56	13.97	28.54	42.51	0.19	49	9.62	8.24	17.86	1.71
63	19.89	36.92	56.81	0.71	64	11.79	9.45	21.24	1.79
77	31.28	50.07	81.35	0.87	77	12.85	10.13	22.98	1.55
91	43.19	64.75	107.94	0.81					

Note: data in the table are the average of five replications.
He et al. (1999).

equivalent to 49%, 47.5%, and 46.5% of the total N, respectively. The averaged ratio of the entire period was 0.89 with NH_4^+–N equivalent to 47.2% of the total, and thus, proso preferred NO_3^-–N.

The absorbed ratio of buckwheat varied from 0.87 to 1.79, and it increased with progress of plant growth. The averaged ratio before flowering (before day 35) was 0.99, and the absorbed NH_4^+–N occupied 49.8% of the total N; from flowering to maturing, it was 1.71, and NH_4^+–N occupied 63.1% of the total N. Obviously, before flowering, buckwheat took up equal amounts of either N form, and after flowering, NH_4^+–N was the main absorbed form. Over the entire period, the averaged ratio was 1.27, and the cumulatively absorbed NH_4^+–N was 55.9% of the total, and thus, buckwheat preferred NH_4^+–N.

The ratio of the tested tomato variety, an indefinitely growing type, also significantly varied with growth stages. Before flowering (grown for 42 days), the averaged ratio was 1.23 and NH_4^+–N occupied 55.1% of the total N; from flowering to fruiting, it varied from 0.79 to 1.02 with 0.89 as an average, and the absorbed NH_4^+–N occupied 47.2%. At fruit maturity of the third anthotaxy (flower head), its cumulatively absorbed NH_4^+–N was 47.5% of the total with an averaged ratio of 0.90. Obviously, at the vegetative growing stage, tomatoes absorbed more NH_4^+–N than NO_3^-–N, while at the reproductive growing stage, the absorbed NO_3^-–N was slightly higher than NH_4^+–N, the degree depending on the fruiting duration period.

Pakchoi (*Brassica chinensis* L) absorbed NO_3^-–N as the main N source in its entire growth period, and the absorbed ratio varied from 0.19 to 0.93 with an average of 0.67, the cumulative NH_4^+–N being 40% of the total.

Obviously, in such a solution with the coexistence of NH_4^+–N and NO_3^-–N at equal amounts, the preference of the six crops to NH_4^+–N or NO_3^-–N varies greatly with the growing stages. Wheat prefers NO_3^-–N at the early stage, while NH_4^+–N at late stages. Maize prefers NH_4^+–N at the seedling stage, but it absorbs almost the same amount of either at the heading stage, while it dominantly takes up NO_3^-–N at flowering and seed formation stages. Buckwheat absorbs almost the same amount of NH_4^+–N and NO_3^-N before flowering and after flowering and mainly takes up NH_4^+–N. Tomatoes mainly took up NH_4^+–N at the vegetative stage, and more NO_3^- at the reproductive stage. Proso and pakchoi in their entire life cycle absorb NO_3^-–N with pakchoi being more striking. Although the relative biomass obtained by the two N forms for the six crops is consistently in agreement with their absorption kinetic parameters (*V*max and *K*m) of NH_4^+–N and NO_3^-–N at seedling stages, it is not true for the two

parameters to reflect the situation in the entire lives of the crops. Judged by N amounts and N ratios of NH_4^+ to NO_3^- in the entire lives, wheat, maize, proso, and pakchoi that absorb more NO_3^- than NH_4^+–N belong to NO_3^-–N-preferring crops; buckwheat absorbing more NH_4^+–N than NO_3^-–N belongs to the NH_4^+–N-preferring crop. The preference of tomatoes for the two N forms, on the other hand, depends on the fruiting duration period. Obviously, the entire life responses of the six crops to NH_4^+–N and NO_3^-–N are different from their responses at seedling stages: wheat and pakchoi prefer NO_3^-–N consistently at the seedling stage and also in their entire growing period, and therefore, the seedling results can reflect their entire life responses. For the other four crops, the seedling results cannot give such information on their entire lives.

The uptake ratio of NH_4^+–N to NO_3^-–N at different stages for the six crops is measured under water-cultured conditions, and the results might reflect the real absorptive characteristics of the crops themselves in nature without the disturbance of other factors. Under soil conditions, NH_4^+–N can readily be nitrified, and NO_3^-–N can easily be leached and denitrified, both being kinetic changes. For using the results in practical fertilization, field experiments are needed to determine the amounts and ratios of NH_4^+–N to NO_3^-–N in the soil, and those plant needs so that two N-form fertilizers can be used to regulate the amounts and ratios into suitable ones plants need at different growth stages for their best growth.

6.2. Responses of Rice to NH_4^+–N and NO_3^-–N at Different Growth Stages

Pot and field experiments conducted by early workers have proven that rice is an NH_4^+–N-preferring crop (Prianishnikov, 1945; Wang, 1989). However, its responses to NH_4^+–N and NO_3^-–N at different stages are different. He et al. (1998a) studied its responses to and absorption characteristics of NH_4^+–N and NO_3^-–N at different growth stages using a sterilized water culture and the normal nutritional solution developed by the International Rice Research Institute (Yoshida et al., 1976) in which ammonium nitrate (NH_4NO_3) was used as the N source with a concentration of 1.43 mmol L^{-1} N for each NH_4^+ and NO_3^-. Two healthy plants of the same growing status and of rice seedlings aged 14 days were selected and transplanted in a barrel of 2 L containing the required solution for the entire life (140 days). The solution was changed every 1–2 weeks. The absorption kinetics was measured by the normal depletion method (Sun, 1990; Yang

Table 5.10 The Cumulative Uptake Ratio and Amount of NH_4^+–N and NO_3^-–N of Rice at Different Growth Stages

Growth stage	Days after sowing	Cumulative uptake of N (mg N plant^{-1})			Absorbed ratio (NH_4^+–N: NO_3^-–N)
		NH_4^+–N	NO_3^-–N	NH_4^++NO_3^-	
Transplanting to tillerring outset	14	–	–	–	–
	35	1.87	1.49	3.36	1.26
Tillerring	49	7.29	6.34	13.63	1.12
	56	20.71	11.69	32.40	2.51
	63	41.50	19.64	61.14	2.62
Elongation to booting stages	70	59.37	26.76	86.13	2.51
	84	76.43	43.20	119.63	1.04
Heading to milky ripe-ness stages	98	92.60	59.68	152.28	0.98
	112	110.57	77.20	187.77	1.03
	126	127.63	94.56	222.19	0.98
	140	161.61	128.28	289.89	1.01
Full ripeness stage	154	194.51	152.50	347.01	1.36

Note: data in Table 5.8 are the average of five replications.
He et al. (1998a).

and Sun, 1990, 1991b), and the *V*max and *K*m in Michaelis–Menten equation were calculated.

The results show that the amounts of NH_4^+–N and NO_3^-–N taken up and the ratio absorbed by rice varied with its growing stages (Table 5.10): from transplanting to onset of tillering, the averaged absorbed ratio was 1.15, and the cumulative NH_4^+–N occupied 53.5% of the total N. At the tillering stage (49–70 days after sowing), the averaged absorbed ratio was 2.55, and the uptake amount was 52.1 mg N per plant for NH_4^+–N and 20.4 mg N for NO_3^-–N, the former being 71.8% of the totally absorbed N during the largest period of the absorption of NH_4^+–N. From the tillering to booting stage (70–98 d after sowing), the uptake ratio averaged 1.01, the cumulative NH_4^+–N and NO_3^-–N were 33.2 and 32.9 mg N per plant, respectively, very close to each other. From the heading to milk stage (98–140 d after sowing), the absorbed ratio was also 1.01, equal to the previous stage, the cumulatively absorbed NH_4^+–N and NO_3^-–N were 69 and 68.1 mg N per plant, almost equal. At the dough stage (the stage of wax ripeness), the absorbed ratio was 1.36, and the absorbed NH_4^+–N was 57.6% of the total N. The main reason for the increase in NH_4^+–N

Table 5.11 The Absorption Velocity of Rice Seedlings (14 d aged) for NH_4^+–N and NO_3^-–N

N forms	Absorption velocity (μmol g⁻¹ root FW h⁻¹) at different N concentrations (mmol L⁻¹)							Kinetic parameters	
	0.05	0.1	0.2	0.4	0.6	1.0	2.0	*V*max (μmol g⁻¹ root FW h⁻¹)	*K*m (mmol L⁻¹)
NH_4^+	2.178	4.297	7.857	8.393	9.604	10.113	11.366	12.47	0.189
NO_3^-	1.808	2.600	3.362	4.677	5.682	7.539	8.118	7.92	0.193

He et al. (1998a).

was due to the fact that a large number of the third to fifth tillers were growing vigorously and just in the vegetative growing stage (under field conditions, multiple tillerring was much less, and such a phenomenon seldom occurred.) As a whole for the entire growing period, the absorbed ratio of NH_4^+–N to NO_3^-–N varied between 0.88 and 2.62 with an average of 1.28; the cumulative NH_4^+–N absorbed was 56.1% of the total N. Although rice is a stronger NH_4^+-preferring crop and the cumulative NH_4^+–N was higher than NO_3^-–N, it did not depend on NH_4^+–N. When NH_4^+–N and NO_3^-—N coexisted, the relative amounts of NH_4^+–N and NO_3^-–N absorbed by rice varied noticeably with growth stages. For the vegetative growth period from transplanting to the end of tillerring, rice took up more NH_4^+–N than NO_3^-–N, the NH_4^+–N being 68.9% of the total N absorbed, and the dominant function of NH_4^+–N at tillerring stage was more striking. From elongation to maturity, the ratio of the two N forms approached 1 or so, and such a ratio was stable, showing that it can equally take up NH_4^+–N and NO_3^-–N. In fertilization practice, we should fully use the characteristics of NH_4^+–N and NO_3^-–N uptake at different stages, rationally regulate the ratio in the soil to meet the requirement of rice on the two N forms. Considering the previous results obtained by Prianishnikov (1945), application of NH_4^+–N at an early stage and alternatively applying NH_4^+–N and NO_3^-–N at a late stage may promote rice growth and increase its yield.

Under constant external conditions, the relative uptake of NH_4^+–N and NO_3^-–N by rice is the comprehensive result of internal factors. Such results can be explained by the absorption kinetic parameters. As seen from the two parameters (Table 5.11) determined in the absorption experiments and the amounts of NH_4^+–N and NO_3^-–N absorbed, there is a close relation between them. At the 14-d seedling stage, the root *K*m to NH_4^+–N

and NO_3^-–N both approached 0.19 mmol L^{-1}, showing that there was no difference in the affinity to the two N forms, but the *V*max for NH_4^+–N was 12.47 μmol g (fresh root)$^{-1}$ h^{-1}, while that of NO_3^-–N was 7.92 μmol g (fresh root)$^{-1}$ h^{-1}, the former being much larger than the latter, and thereby NH_4^+–N absorbed was more than NO_3^-–N. Such relations not only provide evidence that rice prefers NH_4^+–N but they also give a preliminary explanation to its preference.

The preference of rice for NH_4^+–N has a physiological and metabolism basis in relation to the NR in plants and the terminal oxidase that functions in root respiration. In a certain range of concentration, the NRA is positively correlated with the NO_3^- concentration. Young roots of rice lack NR that can only be formed gradually through a period of induction of the roots in NO_3^-–N solution. As a result, at the initial period of growth, rice has a low absorbing velocity of NO_3^-–N. With the progress of rice growth and development and increase of NR activities, the absorbing velocity of NO_3^-–N is gradually accelerated. Also, the preference of rice to NH_4^+–N or NO_3^-–N is linked with the types of the terminal oxidase functioning in root respiration (He and Meng, 1987). At the seedling stage, cytochrome oxidase is dominant in roots, and therefore, rice takes up more NH_4^+–N than NO_3^-–N. With plant growth, yellow enzyme gradually becomes dominant, and the absorption of NO_3^-–N correspondingly increases.

6.3. Effect of Media pH on the Uptake of NH_4^+–N and NO_3^-–N of Wheat

As one of the environmental factors, the media pH affects crop growth and nutrient uptake. Too high or too low pH does not only influence crop growth and development but also the relative amount of NH_4^+–N and NO_3^-–N (Barber, 1984a; Hageman, 1984; Haynes, 1986; He and Meng, 1987; Wu and Liu, 1985).

He et al. (1998b) carried out a solution culture experiment using spring wheat as a test crop and adopted the Prianishnikov (1945) solution with equal amounts of NH_4^+–N and NO_3^-–N to study the effect of pH on the relative uptake ratio of NH_4^+–N to NO_3^-–N and crop biomass as well as pH changes caused by the uptake of the two N forms. Concentrations of both N forms were 42 mg N L^{-1}, and three pHs, 5.0, 6.5, and 8.0, in original solutions were studied. For each pH, there were two treatments: solution with plants and that without plants (control). For culturing, 10-d-aged healthy and vigorous plants were selected and transplanted in water-cultured

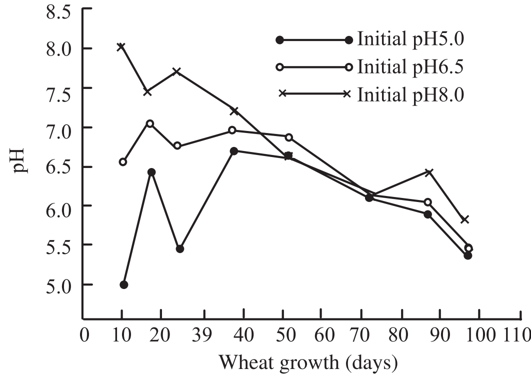


Figure 5.4 Change of solution pH with wheat growth stage. *He et al. (1998b)*

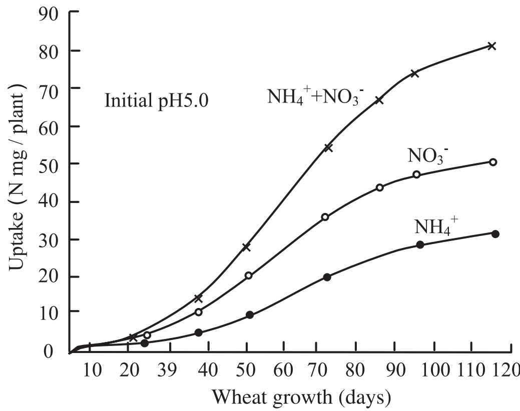


Figure 5.5 Cumulative N amount of wheat at pH 5. *He et al. (1998b)*

pots in which the pH had already been regulated as designed. The normal depletion method was used, the solution was changed every 1–2 weeks, and pH was determined before the solution was changed. The pH measured during culturing is called the growing solution pH, to differentiate it from the initial pH before culture.

The results obtained showed that when NH_4^+–N and NO_3^-–N coexisted in solution, the growing solution pH changed with wheat growing stages (Figs 5.4 and 5.5). The growing solution pH increase when the initial pH was low, while it declined when the initial pH was high. When the initial pH was 5.0, the growing solution pH was consistently higher than the initial value, and increased by 1.1 units on average during the entire growth period. The increased range was higher at the early than at the late stage. When the initial

pH was 6.5, the growing solution pH changes were relatively small, only a 0.3-unit increase before heading (before 60 d after transplanting), while a 0.7 unit decrease at the late period with an average of 0.5 units. When the initial pH was 8.0, the growing solution pH gradually declined with plant growth, the averaged decline being 1.3 units. No matter whether the initial pH was high or low, in the early growing period (before grown for 60 d), the growing solution pH for all pH treatments tended to be neutralized, and changed between about 6.5 and 7.5 while at late stages, the growing solution pH tended toward acidification with changes ranging from 6.5 to 5.5.

The growing solution pH changes were caused by the unequal uptake of the two N forms by wheat plants. With higher NH_4^+–N uptake, the growing solution pH decreased, while with higher NO_3^- uptake, it increased. The low pH benefited NO_3^-–N uptake, but with plant growth, NH_4^+–N uptake was gradually increased, and the high pH benefited NH_4^+–N uptake, but with plant growth, NH_4^+–N uptake was declined. Grown at pH 6.5, wheat took up almost equal amounts of NH_4^+–N and NO_3^-–N.

The N absorbed curves of NH_4^+–N or NO_3^-–N were similar in shape under different pH conditions, but the total amounts of either N form were greatly different (Figs 5.6 and 5.7). At initial pH 5.0, the total absorbed NO_3^-–N was significantly higher than that of NH_4^+–N that was only 38% of the total amount. At pH 6.5, the total absorbed NO_3^-–N was equal to NH_4^+–N. At pH 8.0, the total absorbed NH_4^+–N was significantly higher than NO_3^-–N (only 35% of the total N). As seen from the results, the uptake of NH_4^+–N increased, while that of NO_3^- decreased with pH increase, but the magnitude of decline or increase was greatly different. When the initial pH increased from 5.0 to 6.5, the uptake of NH_4^+–N increased 14.5 mg per plant, while the NO_3^-–N only decreased 1.5 mg per plant; when the pH increased from 6.5 to 8.0, NH_4^+–N only increased 2.8 mg per plant, while the NO_3^-–N decreased 22.2 mg per plant.

The total uptake of NH_4^+–N and NO_3^-–N at different growing stages of wheat varied also with initial solution pH: at pH 6.5, it was the highest, while at pH 5.0 and 8.0, it declined and there was no significant difference in the total uptake between the two pHs.

Similarly, the uptake ratios of NH_4^+–N to NO_3^-–N of wheat varied with the initial pH and growth stages (Table 5.12). At initial pH 5.0, the ratio gradually increased, ranging from 0.27 to 0.87. The averaged ratios at the tillering stage (before day 38), elongation to heading stages (from day 38 to day 87), and from flowering to maturing stages (from day 87 to day 117) were 0.38, 0.63, and 0.81, respectively, with an average of 0.61 for the entire growth period; the

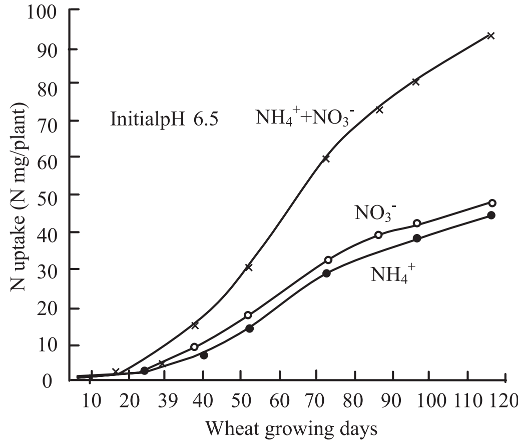


Figure 5.6 Cumulative N amount of wheat at pH 6.5. *He et al.(1998b)*

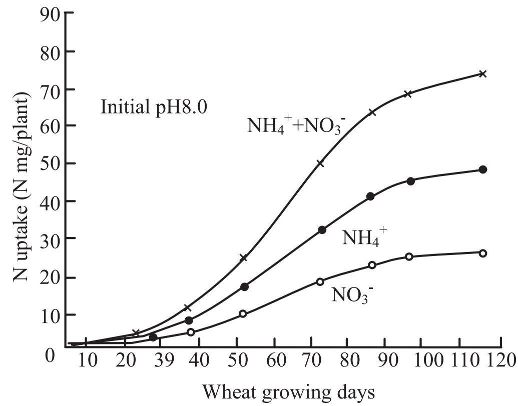


Figure 5.7 Cumulative N amount of wheat at pH 8.0. *He et al. (1998b).*

absorbed NH_4^+–N was 27.6%, 38.7%, and 44.9% of the total N, respectively, with an average ratio of 37% in the entire life cycle. At initial pH 6.5, the uptake ratio also increased with the progress of plant growth, varying from 0.67 to 1.18, significantly higher than that at initial pH 5.0. The averaged ratios at the three stages were 0.79, 0.90, and 1.18, and the uptake NH_4^+–N was 44.2%, 47.5%, and 54% of the total N absorbed, respectively. More NO_3^-–N was taken up at the early stage, while more NH_4^+–N at late stages. As a whole, the averaged ratio of the entire growing period was 0.94, and the total NH_4^+–N absorbed was 48.4% of the total, and the NH_4^+–N was basically equal to the NO_3^-–N amount. At initial pH 8.0, the uptake ratio was higher, ranging from 1.14 to 2.51. The averaged uptake ratios were 2.40, 1.76, and 1.89

Table 5.12 The Total Uptake Amount of NH_4^+–N and NO_3^-–N (mg N per plant) During the Entire Growing Period of Wheat From Solutions at Different pHs

pH	NH_4^+–N	NO_3^-–N	Total
5.0	30.2 b	49.3 a	79.5 b
6.5	44.7 a	47.8 a	92.5 a
8.0	47.5 a	25.6 b	73.1 b

Note: on the basis of variance analysis, LSD (Least Significant Difference) is used for multiple comparisons. The same letter in each column indicates no significance at $P<0.05$.
He et al. (1998b).

Table 5.13 Effect of Nutritional Solution pH on the Absorbing Ratios of NH_4^+–N to NO_3^-–N of Wheat

Growth period of time (days)	Ratio of NH_4^+–N to NO_3^-–N absorbed		
	pH 5.0	pH 6.5	pH 8.0
10			
17	0.27	0.82	2.51
24	0.28	0.67	2.48
38	0.42	0.82	2.36
52	0.56	0.84	1.44
73	0.62	0.94	1.83
87	0.73	0.89	2.02
97	0.78	1.17	1.79
117	0.87	1.18	2.00
Mean	0.61	0.94	1.85

He et al. (1998b).

and NH_4^+–N absorbed occupied 70.6%, 63.8%, and 65.4% of the total N, respectively, with an average of 1.85 in the entire growing period. The total NH_4^+–N amount was 65% of the total N, significantly higher than NO_3^-–N.

The averaged ratios in the entire growth period were 0.61, 0.94, and 1.85 at initial pH 5.0, 6.5, and 8.0, respectively. Again, the results clearly show that the relative amount of NH_4^+–N increased, while NO_3^-–N decreased with an increase in the pH. At pH 6.5, the averaged ratio was close to 1. Obviously, wheat preference to NH_4^+–N and NO_3^-–N was determined first by media pH (Table 5.13).

The initial pH did not only affect the uptake of NH_4^+–N and NO_3^-–N, but only wheat growth and yield. Cultured in the three pH solutions, wheat grew well without any retardation. However, at pH 6.5, the growth vigor, shoot dry weight, total dry weight, seed weight, and seed number per ear and N uptake amount were all higher than in the other two pH treatments, which had the most suitable pH values of the three pHs for wheat growth

Table 5.14 Effect of Nutritional Solution pH on Wheat Growth

Items determined	pH 5.0	pH 6.5	pH 8.0
Plant height (cm)	47.40	47.90	48.20
Shoot dry matter (g/plant)	3.94	4.68	3.76
Root dry weight (g/plant)	0.24	0.21	0.16
Total dry matter (g/plant)	4.18	4.89	3.92
Root/shoot (%)	6.04	4.53	4.36
Seed yield (g/plant)	1.36	1.86	1.21
Seed number per ear	32.40	37.40	30.50

He et al. (1998b).

(Table 5.14). However, the treatment with pH 5.0 was slightly higher than that with pH 8.0 in the total N uptake amount. Root dry weight, root to shoot ratio increased with pH decline. Obviously, although pH 6.5 is more suitable to wheat growth, wheat that can strongly adapt to media pH can normally grow in a wide range of pHs, at least in the range of pH 5–8.

7. EFFECT OF NH_4^+–N AND NO_3^-–N FORMS ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF CROP PLANTS

Different plants have some differences in absorption, transport, storage, and assimilation of NH_4^+–N and NO_3^-–N. Such differences will result in morphological and physiological changes, plant growth, and development (Cechin and Fumis, 2004).

7.1. Effect of N Forms on Crop Root Morphology

Drew et al. (1973) found that the partial supply of NO_3^-–N had increased the number and increased the velocity of the first and secondary lateral roots of barley. Further work showed that the partial supply of NH_4^+–N had also promoted the extension and occurrence of lateral roots, particularly the lateral root density (Drew, 1975), but the lateral roots did not grow in the normal state. Due to proton released out of the cells in accompanying uptake of NH_4^+–N, the rhizosphere is acidified. This might be a reason why the supply of NH_4^+–N did not stimulate but could produce such an abnormal growth of lateral roots. Results of other researchers show that the supply of NH_4^+–N leads to the decline of rhizosphere pH, which inhibits root growth (X.F. Cao et al., 1999; Marschner, 1995). Sattelmacher et al. (1993) found that supply of either NH_4^+–N or NO_3^-–N had stimulated the lateral root

growth of maize, but NO_3^-–N was more striking. Zhang and Forde (1998, 2000) partially supplied NO_3^-–N to a mutant of *A. thaliana* (L.) Heynh and found that the addition of NO_3^-–N had obviously stimulated the first lateral roots extending, while the lateral root density was not affected, and the NRA was totally inhibited. Based on this fact, they postulated that the responses of lateral roots to NO_3^- were not related to the NO_3 assimilative ability, but to its function as an environmental signal. Similar results are also reported by others. Zhang and Forde (2000) found that treating partial roots with NH_4^+ and Gln did not stimulate lateral root growth. All this shows that partial supply of NO_3^-–N to *A. thaliana* may not have a nutrient function but a signal function to stimulate and regulate the lateral root growth and development. Such a result that the partial supply of NH_4^+–N promoted the lateral root extension from the early study might be caused by NO_3^-–N that is formed due to the nitrification of NH_4^+–N.

7.2. Effect of N Forms on Chlorophyll Contents and Photosynthesis

Nitrogen is the main component of chlorophyll, and the application of N fertilizer can lead to the synthesis of plant chlorophyll (Chen and Li, 1983; Oaks et al., 1977; Xie and Chen, 1989). Chlorophyll content increases with the increase in N content in leaf blades. Deficiency of N can bring about the decline in photophosphorylation in chloroplasts (Evans, 1987). Evans (1987) found that the CO_2 fixation ability of wheat leaves was related to N contents of leaves. With increase in the N content in leaves, CO_2 assimilative rate increased; after the N content exceeded a certain level, the assimilative rate decreased. However, the N effect on intercellular CO_2 concentration turned out to be opposite to the conductance. Improvement of N nutrition decreased the leaf intercellular CO_2 concentration, showing that nonstomatal limitation might be the major factor that affects photosynthesis and N influence.

Different N forms have a different impact on the chlorophyll content of crop leaf blades (Yang and Sun, 1990) and photosynthetic rate (Sun, 1990; Xiao et al., 2000; Yang and Sun, 1990). Xiao et al. (2000) reported that for coexistence of NH_4^+–N and NO_3^-–N, both chlorophyll content and the net photosynthetic rate are the highest, followed by NO_3^-–N, while NH_4^+–N is the lowest.

7.3. Effect of N Forms on N-Containing Compounds

In plants, NO_3^-–N can be reduced in the roots and leaves. In cells, NO_3^-–N is mainly stored in vacuoles (Martinoia et al., 1981). The NO_3^-–N stored in

vacuoles can be release to the cytoplasm. Martin (1973) and Rufty et al. (1982) postulated that the release velocity was the limiting step for NO_3^-–N reduction. Plants can use the stored N during the growth period. After reduction in plants, NO_3^-–N can further be assimilated into N-containing compounds.

Deficiency of N can result in the decline of enzyme proteins and NO_3^-–N concentration. The decline in NRA may be affected by the feedback inhibitive reaction of the product, NH_4^+, which is possible to inhibit NRA (Lauchli and Bielaski, 1983).

There are different results that show the effect of NH_4^+–N and NO_3^-–N on crop N contents and N components. Some researchers report that on supplying NH_4^+–N, the total N, free amino acids and amides are higher than in the case of NO_3^-–N (Hageman, 1984; Weissman, 1964). However, others report that the two N forms do not have a great influence on crop N accumulation and N-containing components.

Cao and Li (2003a) studied the effects of N forms on N-containing substances, biomass, and physiological characteristics of the leaves and roots of wheat at the coprogressed stage of vegetative and reproductive growth using water culture. The results show that for the ratio of 50:50 of NH_4^+–N to NO_3^-–N, the N-containing compounds and the total amount of chlorophyll, amides, and NO_3^-–N are the highest, and so are leaf areas and root activity; this results in the highest biomass and seed yield (Table 5.15).

7.4. Effect of N on Carbohydrate and Nutrient Accumulation

Nitrogen is not only the most important structural substance but also the most active and main component existing in all enzymes (Zhao et al., 1998). The supply of N and N forms does not only affect carbohydrate formation and accumulation but it also affects the uptake of other nutrients.

Nitrogen forms influence the carbon metabolism of plants (Cramer et al., 1993). Smith (1993) reported that the application of NO_3^-–N exerted a greater effect on carbon assimilation in photosynthesis than NH_4^+–N did, but Cruz et al. (1993) reported that NO_3^-–N nutrition benefited the accumulation of sucrose while NH_4^+–N benefited starch accumulation. Mckee (1962) pointed out that full supply of NO_3^-–N could have increased plant organic acids, while the full supply of NH_4^+–N could have consumed large amounts of organic acids and carbohydrates, and the main carbohydrate was starch, not sugars although sugars were also influenced. C.X, Dong et al. (2004) found that with replacement of NO_3^-–N by NH_4^+–N , tomato growth and organic acids were correspondingly changed. Kirkby (1968) reported that compared to NO_3^-–N, the use of NH_4^+–N reduced sugar content by 30%

Table 5.15 Effect of N Forms on Some Physiological Characteristics of Wheat During Middle and Late Growth Stages

Items determined	N forms (NH_4^+–N:NO_3^-–N)					
	100:0	**75:25**	**50:50**	**75:25**	**0:100**	**Urea**
	Root activity ($mgTTC\,g^{-1}\,FW\,h^{-1}$)					
	1.055	0.976	1.271	1.132	0.907	1.050
	Chlorophyll ($mg\,g^{-1}\,FW$)					
A	0.915	0.878	0.934	0.924	0.967	0.921
B	0.643	0.557	0.825	0.576	0.618	0.599
Total	1.647	1.435	1.759	1.500	1.585	1.520
	NO_3^- N content ($\mu g\,g^{-1}\,FW$)					
Leaf blade	351.1	134.6	148.1	99.8	133.4	139.0
Roots	69.3	135.3	215.0	179.4	111.2	65.6
	Amide N content ($\mu g\,g^{-1}\,FW$)					
Leaf blade	116.3	304.6	397.1	481.5	352.7	662.7
Root	586.3	545.3	1024.6	534.8	650.5	630.7
	NH_4+–N content ($\mu g\,g^{-1}\,FW$)					
Leaf blade	377.3	273.1	317.9	320.8	299.9	258.1
Root	24.2	24.2	27.9	29.2	29.5	40.8
	Fresh weight ($g\,FW\,plant^{-1}$), dry weight ($g\,DW\,plant^{-1}$), and leaf area ($cm^2\,plant^{-1}$) of the aboveground part					
Fresh weight	6.59	7.8	8.25	9.05	8.35	7.56
Dry weight	1.72	2.1	2.12	2.16	2.54	1.87
Leaf area	112.7	155.5	181.61	174.30	160.41	91.50
	Root dry weight ($g\,pot^{-1}$), root length (cm), and shoot dry weight ($g\,pot^{-1}$) and seed weight ($g\,pot^{-1}$)					
Root weight	3.6	3.15	3.6	3.53	3.5	3.7
Root length	27.7	21.90	20.8	19.1	19.1	20.0
Shoot weight	61.8	58.11	73.6	51.9	57.6	62.4
Root/shoot	0.058	0.054	0.049	0.068	0.061	0.060
Seed weight	25.38	16.58	27.72	22.14	16.26	17.81

Cao and Li (2003a).

in plants. The reason for the reduction of organic acids and sugar content by NH_4^+–N application probably relates to the reaction difference in the assimilation and metabolism of NH_4^+–N and NO_3^-–N (Hageman, 1984). Providing carbon atoms and energy can promote the assimilation of NH_3, and high carbohydrate contents in plants can in turn promote NH_3 absorption (Mengel and Kirkby, 1987). The influence of NH_4^+–N and NO_3^-–N on organic acids and carbohydrates may also relate to the N-supplied rates that affect N concentration (Hageman, 1984). Xu et al. (2005) found in studies of muskmelon (*Cucumis melo*) that different combinative ratios of N

forms had affected the carbohydrate types and its distribution in different organs of plants. C.D. Li et al. (2003) reported that the NO_3^-–N to NH_4^+ –N ratio had influenced carbohydrate types, and different N forms (mineral N and organic N) had different effects on carbon metabolism of plants. Cao and Li (2003b) found that the use of $NO^-{}_3$–N alone had increased the total soluble sugar amount, reductive sugar accumulation, and the structured polysaccharides contents (cellulose) in roots, stems, and leaves of maize seedlings. Plants grown in NH_4^+–N-supplying media need more carbohydrates for detoxification of NH_3, and thus, plant growth is affected by the reduction of carbohydrates (Givan, 1979). Wild plants grown under low temperatures, arctic desert areas can more easily take up organic N (Jones et al., 2005).

In addition to influencing carbon metabolism, NH_4^+–N and NO_3^-–N also affect the mineral ion uptake by plants. In the process of uptake of ions, plants and the media for plant growth need to maintain their electroneutrality. Due to different rates of nutrient ions uptake by plants, the accumulation or degradation of the unvolatilized acids may be an effective way for plants to reach electroneutrality. Most organic acids exist in vacuoles in anion form, and are therefore slightly acidic (He and Meng, 1987; Radin et al., 1982; Yin et al., 1992). N forms also affect nutrient accumulation in plants (He et al., 1998b; Tal et al., 2001). Cao and Li (2003b) revealed that by providing NO_3^-–N nutrition to maize seedlings, the N accumulated in organs was increased, and P and K uptake amounts were increased. On application of NO_3^-–N, oat plants contained higher K^+, Ca^{2+}, and Mg^{2+} cations (Fan et al., 2005b), while Cl^- and SO_4^{2-} absorption was obviously inhibited (Engels and Marschner, 1993). In contrast, on application of NH_4^+–N, plants contained more anions, such as Cl^-, SO_4^{2-}, and $H_2PO_4^-$, while the absorption of K^+ and Ca^{2+} was often reduced, and brought about NH_3 toxicity (Ikeda and Osawa, 1983), and thus affected plant growth. Tal et al. (2001) found that in equal amounts of N, the reduction of the ratio of NO_3^-–N to NH_4^+–N could increase the N uptake by pepper (*Piper* spp.), whereas it could significantly decrease the absorption of K and Ca. Qiu and Zhang (2003) reported that the N forms affected the growth and N uptake of wheat genotypes with different P efficiencies: NH_4NO_3 benefited N contents in plant parts above the ground and N uptake efficiency of wheat. NO_3^-–N could be used as an osmotic regulation substance, and thus benefited cell elongation and plant growth (Cao and Li, 2003b).

Cao and Li (2003b) investigated the effect of NH_4^+–N, NO_3^-–N, and 1:1 ratio of NH_4^+–N to NO_3^-–N on soluble sugar; reductive sugar contents;

and N, P, and K absorbed amounts of maize seedlings using a sand culture. The results showed that no matter what N form was supplied, starch content in different organs was almost the same; soluble sugar was the highest in stems, followed by that in leaves and roots. The total N content in leaves was the highest, while it was the lowest in roots and stems. On supplying NO_3^-–N alone, the total soluble sugar content in roots, stems, and leaves and the reductive sugar and structured polysaccharide (cellulose) content in stems were the highest. The NO_3^-–N did not only benefit the transport of carbohydrates in plants, but also cell division, differentiation and growth, and the ligase activity of carbohydrates. Although the NO_3^-–N contents in different organs were not high compared to that in NH_4^+–N treatment, the absorbed N, P, and K amounts significantly increased, the physiological characteristics improved, and the yield also increased (Table 5.16).

7.5. Effect of N Forms on NRA

The assimilation and metabolism of N is related to a number of enzymes, of which NR is regarded as the first and most concerned for NO_3^-–N metabolism. The enzyme relates to plant absorption and use of N and the formation of N-contained compounds, and it influences crop yield and quality (Guerrero et al., 1980). As mentioned in the previous section, NO_3^-–N in most circumstances is the major N source in soil, and the major N form absorbed by plants. However, plants cannot directly use NO_3^-–N as a component to take part in N metabolism. When NO_3^-–N enters plants, it must first be reduced to nitrite N, and further to ammonia N, and then it participates in a series of activities. The enzyme responsible for transformation of NO_3^-–N into nitrite N is the NR followed by NiR that catalyzes nitrite N into ammonia N. Only in this way can NO_3^-–N be changed into forms such as amino acids and amides that could be used by plants. In the process, the reduction reaction from NO_3^-–N to nitrite N controlled by NR is the rate-determining step and affects the consequent reactions for the synthesis of proteins (Beevers and Hageman, 1969). As a kind of protein, the enzyme is located on the inner side of the cytoplasmic membrane with a large *K*m, short half life, and fast turnover, and its activity maintenance depends on the continuous synthesis of enzyme proteins (Morilla et al., 1973). The NRA is affected by environmental factors such as light, temperature, CO_2, and water, and plant physiological factors and the magnitude of substrate $NO^-{}_3$ in plants (Xu and Ni, 1990a, 1990b). The NO_3^-–N that enters cells induces and stabilizes NR formation (Guerrero et al.,

Table 5.16 Effects of N Forms on Soluble Sugar, Reductive Sugar, Polysaccharides, and Other Components

Nitrogen forms	Root	Stem	Leaf	Root	Stem	Leaf
	Soluble sugar (mg g^{-1} DW)			Reductive sugar (mg g^{-1} DW)		
NH_4^+–N	0.631 b	0.959 b	0.623b	5.507 b	13.223 b	6.592 b
1:1 Ratio	0.584 b	0.749 c	0.598c	6.245 a	12.117 c	8.208 a
NO_3^-–N	0.867 a	1.218 a	0.707a	5.260 b	17.590 a	6.767 b
	Starch content (mg g^{-1} DW)			Cellulose (mg g^{-1} DW)		
NH_4^+–N	11.122	11.315	10.674	65.779 b	68.311 b	67.375 a
1:1 Ratio	10.919	11.122	11.074	66.997 b	68.611 b	67.567 a
NO_3^-–N	11.187	11.749	10.840	68.451 a	71.178 a	65.910 b
	Total N (NO_3^-–N excluded) (mg N g^{-1} DW)			NO_3^-–N content (mg N g^{-1} DW)		
NH_4^+–N	6.153 a	3.436 b	16.877 b	3.159 b	4.320 a	2.164 c
1:1 Ratio	6.529 a	1.153 c	15.341 c	4.015 a	4.461 a	3.307 a
NO_3^-–N	4.272 b	4.250 a	17.059 a	3.158 b	4.025 b	2.747 b
	Total N (NO_3^-–N included) (mg N g^{-1} DW)			N uptake amount per pot (mg N pot^{-1})		
NH_4^+–N	9.312	7.756	19.041	16.762 c	8.531 b	20.945 c
1:1 Ratio	10.544	5.614	18.652	20.034 a	6.737 c	24.248 b
NO_3^-–N	7.430	8.275	20.585	18.575 b	9.931 a	29.709 a
	P content (mg g^{-1} DW)			P uptake amount (mg pot^{-1})		
NH_4^+–N	4.086 c	2.984 a	4.919 b	7.355	6.565	5.411
1:1 Ratio	5.065 b	0.678 c	1.089 c	9.624	1.695	1.416
NO_3^-–N	5.609 a	1.137 b	8.719 a	14.023	3.070	13.079
	K content (mg g^{-1} DW)			K uptake amount (mg pot^{-1})		
NH_4^+–N	6.977 a	21.331 a	26.884 a	12.559	46.928	29.572
1:1 Ratio	5.984 b	19.493 b	25.204 c	11.370	48.733	32.765
NO_3^-–N	4.853 c	18.849 b	25.755 b	12.133	50.892	38.633
	Maize biomass (fresh weight, g pot^{-1})			Maize biomass (dry weight, g pot^{-1})		
NH_4^+–N	16.9	16.1	7.4	1.8	2.2	1.1
1:1 Ratio	19.5	18.1	8.4	1.9	2.5	1.3
NO_3^-–N	24.1	20.2	10.0	2.5	2.7	1.5

Cao and Li (2003b).

1980). As an induced enzyme, with N stress, the changing trend of NR is identical to that of NO_3^-–N, showing its inducing characteristic (C.L. Cao et al., 1999; Gao et al., 1989; Lin et al., 1998). No matter how high the NO_3^-–N level is in the environment, it cannot be reduced by NR present in the cytoplasm or cell membrane until it enters plants. The NRA is sensitive to water, and the effect of water on its activity can be compared with that in the substrate or NO_3^-–N. If the NR enzyme protein amount is small and the activity is weak, the NO_3^-–N taken up by plants does not reduce or the reduced amount is small (Cai and Zhu, 2000; Ferrari et al., 1973; Lin and Tang, 1989; Magalhaes and Huber, 1991; Mengel and Kirkby, 1987; Mengel et al., 1969; Xu and Ni, 1990a, 1990b). Different genotypes of many crops have different NR activities (H. Chen et al., 2000; X.P. Chen et al., 2000; Dalling et al., 1980; Lin et al., 1986), and efforts have been made to improve NRA through plant breeding. However, some researchers have found that the correlation of NRA with seed yield or total amount of N in seeds can be negative or positive, and there is no consistent pattern.

Most plants can reduce NO_3^-–N in both roots and leaves. The rate of reductive amounts is related to various factors, including the rate of N supplied, plant species, and plant age. Commonly, at a low rate of N supplied, the reduced rate in roots is higher; with an increase of NO_3^-–N supply, the reductive ability of roots to NO_3^-–N becomes the limiting factor, and the total NO_3^-–N form getting transferred to shoots is increased (Marschner, 1986). Santoro and Magalhaes (1983) established the relation of NRA to leaf age. The maximum NRA occurred during the highest leaf expansion period, and later on with the increase in leaf areas and leaf age, the NRA decreased and NO_3^-–N correspondingly increased. In root extension area, the NRA was the highest.

Among various factors, the substrate, NO_3^-–N, is the most significant, and therefore the relation of NRA to N rate used is more widely studied (Fang et al., 1979; Gao et al., 1989; Johnson et al., 1976). The NO_3^-–N level in environment and its concentration in plants affect the leaf NRA. Li et al. (2000) show that the application of N can significantly increase the NRA of an entire peanut plant (*Arachis hypogaea*) at the senescence stage, and increase its N assimilation ability, especially for leaves in middle and low positions. Feng et al. (1986) found that at heading, flowering and grain filling stages, the NRA was increased as the N rate increased, and the highest activity occurred at N rate of 120 kg ha^{-1}. Li et al. (2000) show that the application of NO_3^-–N significantly affects NRA in leaves, seeds, and chaffs of wheat.

At each stage of reviving, elongation, booting, heading (ear sprouting), and flowering, the NRA in wheat leaves increased with increased N levels, and the highest period of NRA occurs at the heading stage.

Plants obtain nutrients mainly through root absorption, and root NRA receives more attention as a major biological property. The relation of N forms to NRA has been reported in many publications (Fang et al., 1979; Haynes, 1986; Mengel, 1983; Yang, 1988; Zhao, 1982). The major point is that as an induced enzyme, NRA is determined from the NO_3^-–N concentration in the media. For most plants, in a certain range, the more the NO_3^-–N is taken up, the higher the NRA will be. To a certain extent, the NRA is positively correlated to the NO_3^-–N accumulation in plants. The use of NH_4^+–N as the sole N source can inhibit NRA (Mengel, 1983) while some organic N may have different effects. Oaks et al. (1977) reported that the NRA in maize roots was reduced by NH_3 and amino acids, and the application of mixed amino acids could partially inhibit the induction of root NRA, and this was much clearer in maturing parts. On addition of glutamic acid and asparagine to the culture solution of the mixed amino acids, the inhibitive effect was much stronger; the addition of any amino acid alone or a similar substance alone, such as lysine, canavanine, azaserine, azetidine-2-carboxylic acid, asparagine and Gln, significantly inhibited the induction of NRA, while the addition of arginine could promote the restoration of NRA induction in root tips. From this, it is assumed that amino acids may influence some process of NRA induction (probably absorption, NO_3^-–N distribution, or enzyme synthesis), but not enzyme metabolism.

Since NR is the velocity-determining enzyme in N assimilation, and some findings show that the total accumulated N at the seedling stage of crops is significantly correlated to NRA, NRA in crop leaves is suggested as an index of N metabolism, seed yield, and protein contents for the selection of crop variety and also for N fertilization. However, some research studies indicate that the relation of NRA to seed yield or total N is unstable (Yang and Sun, 1990).

The uptake amount of N by plants cannot be totally assimilated and used, and part of it still remains in its original form in plants, particularly NO_3^-–N (Cai and Zhu, 2000; Ferrari et al., 1973; Lin and Tang, 1989; Mengel and Kirkby, 1987; Mengel et al., 1969; Xu and Ni, 1990a, 1990b). In plants, NO_3^-–N is the safest form for storage, and its accumulation is much more in plants than that of NH_4^+–N. Some reasons have been proposed for explaining the accumulation of NO_3^-–N in plants. The NR

enzyme itself is first concerned. If the amount of NR enzyme protein is small and the activity is weak, accumulation of nitrate will occur in plant body. Deficiency of photosynthetic products is another reason. In this case, imbalance of carbon and N occurs, and the exceeding accumulation of NH_4^+–N or amino acids plays a role in feedback regulation, leading to the decline in NO_3^-–N reduction or lack of the ability to reduce NO_3^-–N (Aslam and Huffaker, 1982; Beevers and Hageman, 1983; Solomonson and Barber, 1990). The third possibility is that the NO_3^-–N enters vacuoles as an osmotic substance in the regulation of osmotic pressure. The vacuole is the largest pool for NO_3^-–N storage and also a place where the NR cannot enter, and thus, NO_3^-–N can remain there for a long time (Aslam and Huffaker, 1982). It is reported that the magnitude of NO_3^-–N-storing pools varies with genotypes (Brégard et al., 2000; Foulkes et al., 1998). For this reason, NO_3^-–N content can to a certain extent reflect plant uptake, utilization, and transport of NO_3^-–N.

7.6. Effect of N Forms on Turgor Pressure

Turgor pressure plays a significant role in the promotion of plant growth (Pan, 2001; Pan and Dong, 1995). One significant function of the turgor pressure is to promote cell volume expansion (Kazuyuki et al., 1997), and the higher the turgor pressure is, the larger the leaf expansion will be (Liu et al., 2002). The magnitude of turgor pressure is determined by water potential and osmotic potential. Zou et al. (2004) used solution culture to study the effect of NH_4^+–N and NO_3^-–N supply on cell turgor pressure of sunflower (*Halianthus annus* L). The results showed that with the supply of NO_3^-–N, the fresh and dry biomass and root biomass, shoot water content, leaf area, and K^+ and Ca^{2+} concentrations in the top spreading leaves were all higher while the shoot/root ratio was lower than that with supply of NH_4^+–N. No difference was found in the osmotic potential between the two N forms although NH_4^+–N decreased the uptake of K^+, Ca^{2+}, and NO_3^- that, as osmotic mediation materials, might affect osmotic pressure. In comparison, NO_3^-–N increased the leaf water potential and turgor pressure, whereas NH_4^+–N significantly reduced water contents, water potential, and turgor pressure. A decrease in cell turgor pressure will result in a decrease in cell volume. The findings are in agreement with the results observed by Walch-Liu et al. (2000) that leaf area reduction of tobacco by NH_4^+–N was not caused by cell number decline, but by cell volume decline. Walch-Liu et al. (2000) also found that NO_3^- was necessary for the synthesis of CTK or kinetin; supply of NH_4^+–N in a short time had led to the deficiency of

NO_3^- and therefore the decline of CTK synthesis might be the main cause for the retardation of tobacco growth. Cao et al. (2008) found that with the increase in NH_4^+–N levels, leaf areas of vegetables were rapidly reduced, and leaf turgor pressure was greatly reduced. When treated with different levels of NH_4^+–N, osmosis-regulating substances in cucumber (*C. sativus*) seedlings were changed, and both leaf water potential and osmotic potential decreased; the reduction of the leaf water potential was greater than the osmotic potential. As a result, the turgor pressure obviously declined, and the correlation coefficient between turgor pressure and leaf blade areas was as high as 0.98. Li et al. (2005) found that under dry conditions, the effect of osmosis-regulating substances in maintenance of the turgor pressure of apple (*M. pumila* Mill.) leaves was not consistent. With supply of NO_3^-–N, the osmosis-regulating substances increased and osmotic potential declined while the turgor pressure was maintained. When treated with NH_4^+–N, vegetable root growth was inhibited, and pressure potential of xylem decreased (Adler et al., 1996). This in turn resulted in the increase of root resistant force for water to enter plants, decline of leaf water contents, and further led to the decline of leaf water potential (Makobe et al., 1998). Also, NH_4^+–N is able to decrease xylem pressure potential of leaf blades (Pill and Lambeth, 1977), increase root resistant force for water entering plants, change the physiological mechanism of water intake and movement, and inhibit water absorption (Quebedeaux and Qzbun, 1973). All this may influence plants to take in water and thus decrease leaf turgor pressure.

7.7. Effect of N Forms on Protective Enzymes

Fridovich (1975) proposed the biological free radical injure hypothesis, considering that the existence of a large amount of free radicals could result in membrane lipid peroxidation, cause the destruction of cell membrane system, and lead to the death of cells at the most serious status. This hypothesis has been widely used as the mechanism of antisenescence.

Oxygen is absolutely necessary for plants to produce energy and maintain life activities. However, it can also produce many reactive (free) oxygen species (ROS) that threaten all organisms (Yan et al., 1999). Plants growing under natural conditions are inevitably affected by a variety of nonbiological or secondary stresses. Under any stress, the energy charge of plant cells is significantly decreased, electron transport chain saturated, and reductive power increased. All this is beneficial for plants to directly or indirectly form excessive ROS or oxygen free radical and thus produce reactive oxygen stress to plants. This is mainly caused by large amounts of superoxide (free)

radicals generated by enzymatic reaction of H_2O_2 and O_2, or the more oxidation form, hydroxyl radical (OH). The ROS is a type of intermediary product of N metabolism and plays a major role in the destruction of the cell membrane system, lipids, proteins and nucleic acids, and other macromolecules (Ke et al., 2003). The ROS, in fact, can be produced also under normal metabolism process (Cai and Chen, 1999), and thus, the oxidation stress induced by accumulation of ROS is a spreading phenomenon. However, under normal conditions, the metabolism of ROS in plants is in a dynamitic equilibrium, while under stressed conditions, low oxygen stress hinders the electron transfer chains in the chlorophyll and mitochondrion, and results in the decrease of energy charge of cells and increase of reductive power as well as the excess of the harmful low charged cations such as $Fe^{2+}$and Cu^{2+} (C.L. Cao et al., 1999; X.F. Cao et al., 1999). A large number of reactive radical accumulations will destroy the balance between radical formation and scavenging, and yield peroxidative products such as $O_2{\cdot}^-$, H_2O_2 and malondialdehyde (MDA). Also, the accumulative free radicals of the ROS can dislocate the antioxidant system, lead to membrane lipid peroxidation and deesterification, increase cell membrane permeability, and destroy cell membrane structure and function (He and Jin, 1999; VanToai and Bolles, 1991; Wang, 1988; Wang et al., 1989). As a result, plants decrease their endurance ability to low oxygen. The MDA amount is a marker for oxidative stress, and it can particularly measure the degree of membrane lipid peroxidation and the contents of the radicals in tissues (Wang, 1997; L.Y. Zhao et al., 2005; P. Zhao et al., 2005; Zhang et al., 1996) and hence is generally used as an index for judging the degree of cell membrane destruction (Yan et al., 1999).

To scavenge ROS, the living organism develops several lines of defense systems that can scavenge the ROS, forming a balance between the radical formation and its scavenging and resulting in very low concentrations of ROS unable to damage plants. Of the systems (Cai and Chen, 1999; H. Chen et al., 2000; X.P. Chen et al., 2000; Li et al., 1997), the so-called antioxidant enzyme systems or protective enzyme systems, consisting of many enzymes and reducing substances in plants, play decisive roles in getting rid of excess free radicals. SOD, catalase, POD (peroxide enzyme), and glutathione reductase are major ones of such antioxidant enzymes. Since McCord and Fridovich (1969) reported the enzymatic characteristics of SOD, great progress has been made in the biological metabolism of ROS around SOD.

In plants, the protective enzyme systems of SOD, POD, and CAT coordinate to display their roles (Fridovich, 1978; Kong, 1984), and prevents ROS

from reaching a level that can damage the normal physiological metabolism and plant growth. The activities of the protective enzymes of SOD, POD, and CAT can be changed correspondingly under different stressed conditions, and such changes are closely related to MDA accumulation.

The protective enzyme systems are affected by different N rates supplied (Medici et al., 2004; Zhang et al., 2006) and NH_4^+–N and NO_3^-–N forms. Application of N to wheat under dry conditions increases the leaf blade CAT and SOD activities and decreases MDA content (Zhang and Shang-Guan, 2007). SOD and CAT mainly exist in membranes and peroxides, and the increase in their activity was the result of substrate induction (Bowler et al., 1992; Jiang and Zhang, 2004). However, application of N may also increases the possibility of ROS production during the electron transfer in carbon assimilation of mitochondrion, and leads to its accumulation in the mitochondrion and thus promotes the rapid expression of SOD and CAT.

Mohammadi and Karr (2001) reported that increase of N levels could significantly reduce MDA contents, and retard peroxidative damage to cytoplasmic membrane. Xiao et al. (1998) reported that N retarded flag leaf senescence of wheat and enhanced the activity of RuBP (ribulose-1,5-bisphosphate), increased the activity of the protective enzymes, and decreased the content of peroxides such as H_2O_2.

The protective enzyme activity is also influenced by N forms. Results of Luo et al. (1985) show that SOD in green leaves probably exists mainly in chloroplast, while in soybean hypocotyl, a nongreen part, SOD is mainly distributed in the cytosol and mitochondrion. Leaf cells and root cells are present in different environments, and thereby they have different physiological functions and different compositions of cell organelles. Leaves contain a mature plastid, chloroplast, while root cells contain only proplastids that have not yet matured. Both the reduction of NO_3^-–N and the assimilation of NH_4^+–N need NAD(P)H that originates from the photosynthesis of chloroplasts or respiration of the mitochondrion. The chloroplast and mitochondrion are just the two main cell organelles for producing ROS (Cao et al., 2003). Thus, it is reasonable to believe that different N forms will unavoidably produce different effects on the ROS levels, antioxidant enzyme activity, and the protective enzyme activities (Li et al., 1997; Polesskaya et al., 2004; Rios-Gonzalez et al., 2002). Li et al. (1997) found that top dressing of NH_4^+–N fertilizers, $(NH_4)_2SO_4$, and NO_3^-–N fertilizers, $Ca(NO_3)_2$, could at different extents increase the activities of SOD and POD of an apple tree plant, and the effect of NO_3^-–N treated was higher than that treated by NH_4^+–N. Zhu et al. (1998a, 1998b) reported that under different light intensity

conditions, supply of NH_4^+–N and NO_3^-–N had no significant effect on SOD and CAT activities in bean leaf blades. It is found that for the supply of NH_4^+–N to tobacco, MDA contents were higher than for the supply of NO_3^-–N, but the SOD and CAT activities were not significantly different between the two N forms. Cao et al. (2003) found that for a mixed supply of NH_4^+–N and NO_3^-–N, the activity of SOD and CAT was the highest in leaf blades, and so were the MDA contents, while the POD activity in leaf blades was the highest in the supply of NO_3^-–N alone. Zhang et al. (2006) studied the effect of different ratios of NH_4^+–N to NO_3^-–N on the biomass and lipid peroxidation and antioxidant enzyme activity in leaves of spinach using a water culture. The results show that an adequate combination of NH_4^+–N and NO_3^-–N could significantly increase spinach biomass. As a whole, at the ratio of 50:50, spinach suffered less stress from oxidation: its biomass was the highest, and the MDA content was slightly decreased compared to NO_3^-–N alone and the leaf SOD, POD, and CAT activities were all higher than in other treatments although the proline content was the lowest. Rios-Gonzalez et al. (2002) found that the supply of NH_4^+–N did not play a key role for SOD activity in the antioxidation process. However, Polesskaya et al. (2004) found that with full supply of NH_4^+–N, the activities of SOD, POD, and CAT in wheat leaves and roots were significantly higher than with supply of NO_3^-–N. Shou et al. (2000) postulate that supply of NH_4^+–N that induced an increase in superoxide radical formation rate, H_2O_2 accumulation and their induced membrane lipid peroxidation may be one of the causes for the retardation of crop growth.

When plants are grown under low oxygen or waterlogging conditions, wide attention has been paid to the physiological and biochemical functions of the external application of NO_3^-–N, and was an attempt to reveal the mechanism of NO_3^-–N. It has been shown that the external application of NO_3^-–N can promote the survival and restoration of plants that were damaged by waterlogging (Arnon, 1937; Jackson and Drew, 1984; Malavolta, 1954; Prious and Guyot, 1985). Under a waterlogged condition, NO_3^- existing in tomato cells can stabilize cell membrane structure (Oberson et al., 1997). Also, low oxygen environments can activate NR in roots, and NO_3^- can replace of O_2, and it becomes the electron acceptor (Kaiser et al., 1999), reduces NADH levels, and revives the NAD^+ in mitochondria and cytoplasm. At the same time, the excessive electrons are consumed, and thus this decreases the production of free radical(s) derived from oxygen and damage of plants (Monica et al., 1994; Methode and Larry, 1999). The low oxygen stress inhibits tomato growth, while the addition of NO_3^-–N fertilizer can reduce its stress (Cuo

et al., 2003). The external NO_3^- under low oxygen conditions could promote an increase in the GABA content of the embryo bud shell of rice roots (Reggiani et al., 1997), and treated with GABA, the activities of antioxidant enzymes of SOD, CAT, and POD in roots and leaves of muskmelon (*Cucumis melo*) were increased, while the contents of $O_2 \cdot^-$, H_2O_2, and MDA decreased (Gao and Guo, 2004). With salt stress, NO_3^- can produce NO (Abir and Rober, 2004; Corpas et al., 2006; Crawford, 1995; Gupta et al., 2011; Ruan et al., 2004; Siddiqui et al., 2011; Wilson et al., 2008), and a suitable amount of NO can induce the increase of SOD and CAT activities in wheat leaves, and decrease the forming-velocity and cumulative amounts of H_2O_2 and $O_2 \cdot^-$ as well as MDA, and promotes the increase of antioxidant substances and proline content, and thereby retards the damage from leaf oxidation of wheat (Yi and Ching, 2004). With waterlogged stress, external NO_3^- input may first increase GABA content or form NO, and then promote the increase of the SOD and CAT activities in the brown wood roots of cherry (*Solanum pseudocapsicum*), and decreases H_2O_2, $O_2 \cdot^-$, and MDA.

Zhu et al. (2005) found that when the urea content reached 1:3 of the total N and NO_3^-–N to urea N reached 1:2 in solution, the POD activity of *V. natans* was the highest, and the plant's ability for removing reactive oxygen was increased, and the structure and function of the protective membrane were normal; therefore, plants can resist environmental stress. With an increase in urea-N to NO_3^-–N ratio in the cultured solution, the plant growth was inhibited, and the POD activity decreased. This shows that *V. natans* had a certain ability for resisting and adapting to the environment stress, but high urea N (salt) stress hindered the plant growth and retarded the resistant ability. With the decrease in the NO_3^- content in solution, its NRA decreased, and when the NO_3^-–N to urea N ratio was <1, the NRA was even lower than the control and thus influenced the plant uptake and utilization of NO_3^-–N.

Cao et al. (2003) investigated the effect of N forms on the protective enzymes of wheat at the simultaneously progressed vegetative and reproductive stages using water culture with four treatments (urea, NH_4^+–N, NO_3^-–N, and 1:1 of NH_4^+–N to NO_3^-–N) at the same N amounts. Results (Table 5.17) show that the N forms affect the balance between the formation and the scavenging of ROS in cells.

With the supply of the same N form, the SOD and CAT activities in leaves are higher than in roots, while the POD activity is higher in roots than in leaves; supply of either NH_4^+–N or NO_3^-–N alone has no great difference in SOD activities. Supplied with urea, SOD activity is low in

Table 5.17 Effect of N Forms on Protective Enzyme Activities and Some Other Physiological Characteristics of Wheat at Later Growth Stages

Items determined	Ratio of NH_4^+–N to NO_3^-–N			
	100:0	50:50	0:100	Urea
SOD activity (μg^{-1} FW h^{-1})				
Leaf blades	857.94	875.39	864.64	848.00
Roots	704.00	717.53	704.62	668.57
POD activity (μg^{-1} FW h^{-1})				
Leaf blades	2.29	2.16	3.25	1.78
Roots	4.33	4.05	3.71	3.82
CAT activity (μg^{-1} FW min^{-1})				
Leaf blades	488.0	738.9	601.1	779.5
Roots	44.8	11.8	9.1	11.2
MDA content (μmol g^{-1} FW)				
Leaf blades	6.431	7.125	5.233	6.085
Roots	8.721	8.672	9.828	7.515
Soluble protein content (mg g^{-1} FW)				
Leaf blades	20.297	25.768	25.518	33.382
Roots	0.332	0.554	0.378	0.667
Root activity (mg TTC g^{-1} FW h^{-1})				
Roots	1.055	1.271	0.907	1.050
Redox activity in root surface (μmol g^{-1} FW h^{-1})				
Roots	26.24	34.99	19.44	34.99

Cao et al. (2003).

both leaves and roots, while the CAT activity in leaves is the highest. In the photosynthetic process, leaves simultaneously conduct photorespiration, and in roots, there are no mature plastids and only dark respiration occurs. Both photorespiration in leaves and respiration in roots can easily produce H_2O_2 (X.M. Du et al., 2001), and the main function of CAT is to scavenge H_2O_2. Perhaps for this reason, with the supply of urea, the CAT activity in wheat leaves is the highest, while when treated with NH_4^+ the CAT activity in the roots is the highest. Supplied with NO_3^-–N, POD activity in leaves is the highest and MDA contents are the highest in roots, but is the lowest in shoots. Treated with NH_4^+–N, root POD and CAT activities are the highest and the MDA contents in roots the second highest (only lower than both NH_4^+–N and NO_3^-–N). Considering the poor growth, low shoot dry weight, and seed yield, it seems that the NH_4^+–N nutrition has made plants to suffer more stress compared to that for NO_3^-N. The higher MDA may be caused by too much ROS produced in cells that cannot be effectively scavenged by protective enzymes, and on the other hand, by the shortage

of soluble proteins for the formation of protective enzymes. Therefore, even POD and CAT activities are the highest in roots, the enzyme amount is limited, and the large number of ROS in roots cannot be effectively scavenged, making MDA to be relatively accumulated. The accumulated ROS can oxidize proteins (Peng et al., 1998), and the oxidized proteins can very easily be decomposed by the proteinase catalyst (Davies, 1987), further leading to a reduction of proteins. For these reasons, the supply of NH_4^+–N may bring about a vicious cycle for the microenvironment in plant cells. Besides, NH_4^+–N is thought to be unable to move to leaves, so the majority of it must be reduced in roots (Zheng, 1986). This may be another reason for plants to grow poorly.

The supply of equal amounts of NH_4^+–N and NO_3^-–N in solution is another situation. In this case, SOD activity and soluble protein contents in both leaves and roots, root activity, and reductive ability of $K_3Fe(CN)_6$ in root surface areas, shoot dry weight, and seed yield at maturity are the highest among all treatments, while the root to shoot ratio is the lowest. Unfortunately, the MDA contents, products of membrane peroxidation, in leaves are also increased to the highest level. This showed that although as a whole the simultaneous supply of NH_4^+–N and NO_3^-–N produced the best results of all treatments, the highest MDA indicated that the membrane lipid peroxidation was unavoidable at late stages, and plants were limited in their ability to scavenge all the free radicals despite the damaged magnitude of this treatment being not as serious as other treatments.

Obviously, during this wheat growth period, the main enzymes for scavenging the free radicals were different. It was likely that SOD, CAT, and POD in leaves were working together to scavenge the free radicals, while in roots due to the absence of mature plastids and only existence of dark respiration, SOD and POD might be the main enzymes for scavenging the free radicals (X.M. Du et al., 2001).

7.8. Effect of N Forms on Hormones

The rate of vegetative and reproductive growth of higher plants is closely associated with the hormone balance in plants (Peng et al., 2003). In addition to N supplying levels, the different N forms of NO_3^-–N and NH_4^+–N result in a difference in plant growth and development (Lee and Jose, 2003), nutrient uptake, and N metabolism may associate with their different effects on the endogenous hormones that further lead to the changes in growth and development of plants. The hormone levels are affected by many factors, of which N nutrition significantly influences its synthesis, transport

and balance (Davies et al., 1994; Qiu and Hu, 2002; Wang et al., 2005). NH_4^+–N and NO_3^-–N may affect the formation and activity of hormones and thus inhibit or promote cell elongation and plant growth (Plamer et al., 1996; Walch-Liu et al., 2000).

Hormones produced in all multicellular organisms are a type of chemicals released by a cell or a gland. The chemicals, functioning in essence as chemical messengers, can transport or send out chemical messages or signals from one cell to the other or from one part of the body to other parts of the organism through which cell metabolism is affected. Only a small amount of hormone is required to alter cell metabolism. Plant hormones or plant growth substances also known as phytohormones exist widely in plants. The most important include auxins, abscisic acid (ABA), and CTKs.

Auxins, a class of plant hormones with some morphogen-like characteristics, are essential for plant development and have a cardinal role in the coordination of many growth and behavioral processes in the plant's life cycle. Of auxins, the most important is IAA, a potent, native plant-existing heterocyclic compound (Simon and Petrášek, 2011). Derived from indole containing a carboxymethyl group (acetic acid) in molecules, IAA is predominantly synthesized in rapid cell division tissues such as apex (bud) cells of stems and developing fruits and very young leaves of the upper part of plants. Mature leaves and root tips can also synthesize a small amount (Zhao, 2010). When synthesized, it is transported via the phloem to the roots. As an endogenous hormone, IAA plays a significant role in root emergence and elongation and participates in a number of plant activities, including cell elongation, development of the embryo, leaf formation, phototropism, gravitropism, apical dominance, fruit development, abscission, root initiation and development, and the shade-avoidance effect.

Nitrogen forms have an obvious effect on plant IAA contents. Compared to NH_4^+–N, NO_3^-–N significantly promoted rice root growth due to its promotion of the rapid synthesis and transport of root-originated hormone CTK and auxin IAA (Revsbech et al., 1999). Guo et al. (2006) found in a water culture experiment that different N forms had different effects on root hormones of tobacco: the IAA content of tobacco seedlings grown in 100% NO_3^-–N solution was higher than of those grown in 50% NH_4^+–N + 50% NO_3^-N solution. From experiments, it was also found that the auxin concentration in the parts of plants above the ground cultured by NO_3^-–N was higher than that by NH_4^+–N.

Recently, some research studies show that NO_3^- may directly act as signal substance to control lateral root growth. NO_3^- must be reduced

through NR assimilation and then be used by plants, playing its nutritional function. However, the supply of NO_3^-–N to *A. thaliana* in which the NRA was completely inhibited could still stimulate its lateral root growth (Zhang and Forde, 1998). This shows that the responses of lateral roots to NO_3^-–N do not relate to plant assimilation ability of NO_3^-–N, and this gives an indirect support to the view that NO_3^-–N may be an environmental signal substance. The use of N-1-naphthylphthalamic acid to inhibit auxin transport from shoots to roots significantly reduced the stimulating function of NO_3^-–N to the lateral roots of *A. thaliana*. This shows that NO_3^- might influence lateral root growth through the regulation of auxin synthesis and transport (Reed et al., 1998). In the investigation of the IAA-inhibited mutant of *A. thaliana*, Zhang et al. (1999) found that with the partial supply of NO_3^-, the rate of lateral root elongation of one mutant, AXR4, did not increase. From the result, they postulated that the existence of AXR4 could inhibit IAA to display its role, and thus the presence of NO_3^- had no function in stimulating lateral root growth. This suggests that the NO_3^- function in the regulation of lateral root growth should be implemented by IAA.

ABA, a very important endogenous hormone in plants, is mainly synthesized in shoots and transported to the roots through the phloem. The production of ABA is accentuated by stresses such as water deficit and freezing temperatures. As a single compound unlike auxins, gibberellins, and CTKs, ABA functions in the promotion of many plant developmental processes though it has long been thought to play inhibitory roles mostly in bud dormancy and make root and leaf senescence. Peuke et al. (1994) confirmed the function in the closure of leaf stomata by spraying ABA and concluded that ABA increase might inhibit plant growth. The significant role of ABA in the emergence and elongation of lateral roots is specially noted.

The leaf senescence of plants due to N deficiency is thought to relate to the formation of ABA. In water-cultured experiments, some researchers found that for the use of NH_4^+ as the sole N source, leaf growth was inhibited. Such inhibition was thought to be due to an increase in the ABA content in plants (Peuke et al., 1994; Zdunek and Lips, 2001). Peuke et al. (1994) sprayed NH_4^+–N on oilseed plants, such as rape or canola (*Brassica napus*) and palma-Christi (*R. communis*), and found that the ABA content in plant xylem was increased three times compared to when spraying NO_3^-–N due to NH_4^+–N promoting ABA distribution in roots and therefore resulting in the ABA content increase in the xylem. The increase in ABA was considered to relate to plant growth inhibition (Peuke et al., 1998). Wang et al. (2002b) found that when treated with

NH_4^+–N, water transpiration amount was even decreased compared to in plants without NH_4^+–N treatment, indicating that the partial stomata of plants were closed.

As adenine derivatives in chemical forms, CTK, a type of phytohormones mainly produced in roots and transported via the xylem to the parts above the ground, are involved primarily in cell division (cytokinesis), differentiation and expansion, apical dominance, axillary bud growth, leaf senescence, substance movement, and biological synthesis of chlorophyll of yellowish or chlorophyll-reduced cotyledons in the presence of light (Dieleman et al., 1997). Their effects were first discovered by the use of coconut milk in the 1940s by Folke Skoog, a scientist at the University of Wisconsin–Madison (Kieber, 2002). Two types of CTKs were found: the adenine-type represented by kinetin, zeatin, and 6-benzylaminopurine, and the phenylurea-type such as diphenylurea and thidiazuron. The total amount of CTK generally includes three substances: isopentenyladenine (iPAs), zeatin (Z or ZT) or zeatin riboside (ZR), and dihydrozeatinriboside. The majority of adenine-type CTKs are synthesized in roots (Campbell et al., 2008); cambium and other actively dividing tissues are also sites for CTK biosynthesis (Chen et al., 1985). There is no evidence that the phenylurea CTKs occur naturally in plant tissues (Mok and Mok, 2001). CTK is involved in both local and long-distance signaling, the latter of which has the same *in planta* transport mechanism as used for transport of purines and nucleosides (Sakakibara, 2006). Typically, CTK is transported within the xylem and acts in concert with auxins (Campbell et al., 2008). The metabolism of CTK is very fast in aboveground plants, and its decomposition increases with leaf senescence (Dieleman et al., 1997).

The metabolism, transport, and translocation of CTK are regulated by N nutritional levels and N forms. It has been proven that the CTK concentration in plants is positively correlated to N supplied levels (Beck, 1996; Takei et al., 2001; van der Werf and Nagel, 1996). In addition to playing a nutrient role, N promotion of plant growth and development is also through affecting endogenous hormone levels, and the degree of N levels controls the synthesis and transport of hormones, especially CTK (Qiu and Hu, 2002). Under N stress conditions, the synthesis of CTK in roots and its transport to shoots are reduced. This decreases the CTK contents in aboveground parts, leading to leaf senescence at early stages. The endogenous hormones regulate N metabolism and translocation in plants, and thus affects the N use efficiency of plants. Some researchers show that the shoot-to-root ratio changes induced by N-supplying levels are related to CTK (Schiefelben and Benfey, 1994), and postulate that CTK may be the signal substance showing

the N supply status of plants and regulating the relative growth rate of shoots to roots, and may therefore play a role in regulating N metabolism and maintaining the balance of N and C.

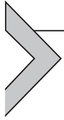
Different N forms affect endogenous hormone levels in plants (Druege et al., 2001) and exert different effects on types of CTK (Gao et al., 1992). Supplied with NH_4^+–N to plants, the transport of CTK from the roots to the shoots is reduced, and the shoot growth of barley and maize is rapidly inhibited (Samuelson and Larsson, 1993; Takei et al., 2001, 2004). This shows that CTK synthesis and transport from the roots to the shoots occurs mainly in environments where NO_3^- exists. Bubán et al. (1978) found that the application of NH_4^+–N produced more CTK in apple trees than when NO_3^-–N was used. However, experiments on tobacco (Singh et al., 1992), Nettle (*Urtica fissa E. Pritz*) (Wagner and Beck, 1993), barley (Samuelson and Larsson, 1993), and maize (Takei et al., 2001) showed that NO_3^-–N promoted the synthesis of CTK more significantly than NH_4^+–N. Takei et al. (2001) suggested that the Z type (Z and ZR) of CTK had been the transferring signal to the aboveground part from roots that earlier sensed changes of the N levels in environments, and NO_3^-–N promoted their synthesis (Parkash, 1982; Samuelson et al., 1992). Walch-Liu et al. (2000) revealed that with the supply of NO_3^-–N to tobacco, Z + ZR contents in the plant xylem and leaf tissues were significantly increased, and this suggested that the inhibition effect of the single NH_4^+–N nutrition to tobacco plant growth would be caused by the decline of Z + ZR concentration in plant xylem sap and leaf tissues. Walch-Liu et al. (2001) also found that with the supply of NO_3^-–N to tobacco roots, the shoot growth was not inhibited due to the increase of the CTK concentration in roots and its transport to shoots. They pointed out that NO_3^-–N was needed by plants to maintain the growth balance and the physiological balance of CTK between shoots and roots and the transport of CTK from roots to shoots in the process for formatting the normal morphology, whereas NH_4^+–N inhibition of plant growth was probably related to the decrease of CTK in roots. Walch-Liu et al. (2001) further found that the aboveground part growth of tobacco had not been inhibited by the supply of NO_3^- to a part of the roots. When grown under the NO_3^--supply condition, root CTK concentration increased and could transport to the aboveground part for maintaining crop growth. Rahayu et al. (2005) also found that NO_3^- treatment increased the Z and ZR levels in plants and promoted plant growth, while NH_4^+ treatment decreased their concentrations in leaves, inhibited plant growth, and this was not related to the changes of isopenteneyl and isopenteneyladenine (iP + iPA) and ABA

concentrations. A study on *A. thaliana* (L.) Heynh showed that NO_3^-–N could increase gene *IPT*3 expression than NH_4^+–N could, and in the *IPT3* mutant, CTK content was sharply increased (Takei et al., 2004). This indicates that *IPT*3 is the key factor responding to the NO_3^- signal, and it thus promotes the synthesis of the CTK. For supply of NH_4^+ to plants, CTK transported from roots toward shoots was reduced, and thereby the shoot growth of barley and maize was rapidly inhibited (Samuelson and Larsson, 1993; Takei et al., 2001). This reveals that the formation and transport of CTK to the aboveground part require NO_3^-.

The application of NO_3^- not only stimulates the biological synthesis and transportation of CTK to the aboveground part but it also stimulates the CTK activity. For example, Z-O-Glucosides or Z-O-xylosides (ZOG) is a kind of CTK with a very low activity. However, in environments with NO_3^-, ZOG is activated and becomes a kind of CTK having a very high activity (Takei et al., 2001). Rice studies show that application of NO_3^-–N at the reproductive stage can increase the endogenous zeatin content and reduce ABA levels more significantly than NH_4^+–N does (Marschner, 1986; Yang and Sun, 1990).

In hormone studies, potato has received more attention than other crops have. The development of potato tubers is a complicated process affected by many factors that are functioning through the regulation of the contents and balance of endogenous hormones (Vreugdenhil and Struik, 1989). A large number of studies have shown the importance of the expression of endogenous hormones on the development of potato tubers (Bouth, 1963; Hu and Jiang, 1992; Lian et al., 2002; Liu et al., 1995, 1997; Ma et al., 2003). It has been shown that gibberellin can promote stolon formation at early stages of tuber formation, while it inhibits tuber formation at late stages (Bouth, 1963; Vreugdenhil and Struik, 1989). Thus, the contents of gibberellin can reflect stolon and tuber occurrence and development to a certain extent, while ABA has a function in the promotion of tuber occurrence (Liu et al., 1997); zeatin (Z) amount can more or less reflect cell division ability. The development of potato tubers does not only relate to the absolute amount of hormones but also to the balance among them, especially the balance between substances that may promote or inhibit plant growth (Meng et al., 1994; Xu et al., 1998). In the process of potato tuber development, the ratio of IAA to ABA reflects a relation of promotion and inhibition of the two factors in the tuber expansion period. Of the factors that can be controlled for potato yield and quality, the nutrient factor is the simplest and the most important. The effects of nutrient factors on hormone levels have been

investigated in cotton, maize, and rice (Liu et al., 2006; Ma et al., 2006; Nie et al., 2006; Wu et al., 1998; L.Y. Zhao et al., 2005; P. Zhao et al., 2005), but no result has been reported in potatoes at least in our survey range.



8. CONCLUSIONS

1. The NH_4^+ and NO_3^- ion uptake by plants is >70% of the total cation and anion amount taken up in plants. The NO_3^- nutrition in plants was realized before N was discovered as an element, while the NH_4^+ nutritional role was discovered later by many scientists.
2. Soils contain a large amount of organic matter and 90% N in the soil is in organic form. The intermediate products of soil organic matter, metabolic products of microorganisms, and plant root secreted substances can be absorbed by plants, particularly the simple organic N compounds such as amino acids. Organic N nutrition affects plant product quality and plant metabolism, depending on organic N forms and molecular structure, concentrations, and plant species.
3. The organic N passes cell walls and arrives at the plasma membrane through the apoplast and cytoplast systems and may, in addition to endocytosis, get transported across the plasma membrane by an active process (sugar/proton cotransport) or a passive process that may relate to ion channel (Zhang et al., 2001, 2002). When taken up by plants, simple organic N compounds, such as amino acids, can be rapidly assimilated and transformed into other amino acids by transamination and deamination.
4. The Michaelis–Menten equation and its two parameters, Vmax and Km, have been widely used to measure the ability and efficiency of roots absorbing the two ions by crop plants. The uptake amounts of both NH_4^+–N and NO_3^-–N at the plant seedling stage are well in agreement with the two parameters, particularly at low concentrations, but are not fully in agreement with the uptake of the two ions during the entire growing period of crops.
5. In addition to the direct uptake by root interception, NH_4^+–N and NO_3^-–N can move from bulk soil to the root surface by mass flow and diffusion. All these are directly or indirectly related to soil water. Diffusion is more important to NH_4^+–N, while NO_3^-–N movement mainly depends on mass flow.

6. Roots are the major organs for the uptake of nutritional ions. On arriving at the root surface, NH_4^+ and NO_3^- passively enter the root epidermis cell wall that is permeable and allow the entry of water, air, and solute particles through the symplast and apoplast and then radically and vertically move across the cortex where the two ions in the apoplast enter the cortex symplast for passing the endodermal Casparian trip to the endodermis. From the endodermis, the two ions go to the stele and empty into the xylem, or flow to the apoplast or get stored in vacuoles in addition to reduction or direct assimilation into amino acids. The movement occurs from cell to cell. Cells are connected by intercellular bridges (plasmodesmeta), and they form a continuum of cytoplasm from one cell to the cytoplasm of adjacent cells. On emptying into the xylem, NH_4^+ and NO_3^- are transported to the shoots via the transpiration stream. Such transport is obviously important throughout the plant to move N nutrients from the source to the sink. Several members of NO_3^- transporters have been found to control the NO_3^- distribution within the shoots.

7. NO_3^-, the dominant form generally present in a higher concentration than NH_4^+ in the soil solution, is usually taken up in large amounts by crops and is readily mobile in the xylem and can also be stored in vacuoles of roots, shoots, and storage organs. The plasma membrane serves as a barrier to the passive movement of NO_3^- ions into and out of the cell. Transportation of NO_3^- across the plasma membrane along the electrochemical gradient is thought to occur by proton–anion cotransport (symport) or exactly the H^+/NO_3^- cotransport, by transport proteins or carriers or by specific ion channels. In addition to the influx of NO_3^- into epidermal and cortical root cells that is essential to plant growth, its efflux back into soil solution can also occur mainly due to excess N supply.

8. NH_4^+ is in equilibrium with NH_3, and in most soils, the pH is considerably low and NH_3 concentrations are usually very low. NH_4^+ uptake through the plasma membrane has been considered to occur in three ways, either active or passive, or both. Passive uptake may occur at the initial stage of uptake while at the second stage active uptake may be predominating. For passive uptake, NH_4^+ ions passing through the membrane is thought to exist in either NH_4^+ or NH_3 form, and this way may be related to the facilitated diffusion through channels that simply allow transport of NH_4^+ across the membrane along the electrochemical gradient. Since NH_4^+

ions resemble K^+ in terms of ionic radius and size of the hydration shell, it may be able to permeate the membrane through K^+ channels. The active uptake includes the H^+/NH_4^+ cotransport, and specific transporters. Three constitutive AMT in roots of *A. thaliana* inducible by N starvation have been found. Throughout the plant, NH_4^+ transport is to a very large extent carried out by members of the NH_4^+ transporter family, especially the AMT1 family. Such an uptake depends much on the carbohydrates in roots. The uptake of NO_3^- and NH_4^+ can induce great changes of rhizosphere pH up to 2 pH units higher or lower than the bulk soil pH.

9. When NO_3^- enters root cells of plants, a large part is reduced and transformed into amino acids and proteins in roots, part may transport to shoots through hadromestome and be reduced there, and/or part may temporarily get stored in vacuoles of roots, stems, and leaves. NO_3^-–N cannot be directly used by plants until it gets reduced to NH_3. The reduction is catalyzed by enzymes in two steps: the first step takes place in the cytoplasm by NR that transforms NO_3^- into HNO_2, and the second step occurs in the chloroplast (shoots) or proplastids (roots) by NiR that converts HNO_2 into NH_3. The importance of reduction and assimilation of NO_3^- for the life cycle of plants is similar to that of the reduction and assimilation of CO_2 in photosynthesis. With the supply of NO_3^- to plants, little accumulation of NO_2^- occurs, indicating that the NO_3^- reduction to NO_2^- is the rate-limiting step for NO_3^- assimilation. For reduction and assimilation of NO_3^-, large amounts of energy and C skeletons are required compared to that for NH_4^+–N. Plants, except those that reduce and assimilate NO_3^-–N in roots, have adopted different measures to save C skeletons. Both roots and shoots are capable of NO_3^- reduction. The uptake and reduction of NO_3^- and the reductive ratios in roots or shoots depend mainly on plant species; carbohydrates in plants; and NRA as well as environmental conditions such as NO_3^- concentration, medium pH, complementary ions, light, and ambient CO_2 concentration. A low soil pH benefits NO_3^- uptake and uptake of NO_3^- will increase rhizosphere pH. Due mainly to NO_3^- uptake exceeding NO_3^- assimilation by plants, a large amount of NO_3^- is accumulated in the vacuoles of roots and shoots at vegetative stages. With the exception of vegetables in which NO_3^- may influence the quality of products, storage of NO_3^- benefits the supply of N nutrients for later growing stages

of plants, and guarantees the N requirement when the medium is reduced in N supply.

10. Ammonia is a central intermediate in plant N metabolism. In addition to transporting to shoots with transpiration stream through the xylem and storing in the vacuoles of roots and shoots, the NH_4^+ entering plant roots can be directly assimilated. Assimilation of NH_3 by plants takes place by the mediation of GS–GOGAT enzyme systems in two steps: with requirement of ATP to add NH_3 to glutamate to form Gln as the first step and transferring the NH_3 from Gln to α-ketoglutarate to form two glutamates as the second step. Therefore, Glu and glutamate serve as the major entry points of NH_3 into organic compounds. Once NH_3 has been incorporated into glutamate, it can be transferred to other carbon skeletons by various transminases to form additional amino acids. The glutamate and Gln formed by NH_3 assimilation can rapidly be used for the synthesis of LMWONCs such as other amides, amino acids, ureide, amines, and peptides. In higher plants, besides other amides, polyamides and betaine, glutamate and aspartate in the amino group, and Gln in the amide group are important organic N compounds of most plants for transfer of N from source organs to sink tissues and for building up reserves during periods of high N availability. Ammonia assimilation in roots has a large requirement for C skeletons. To minimize C costs for root-to-shoot transport, the bulk of the N assimilated in roots is transported in the form of N-rich compounds with N/C ratios being >0.4. There are up to 200 different amino acids in plants, but only about 20 of are required for protein synthesis. Not much is known about the role of a large number of nonproteinogenic amino acids in plants, but at least some of them are important for plant nutrition. The LMWONCs formed in roots will be further synthesized into HMWONCs such as proteins and nucleic acids. Such synthetic processes are extremely important for plant physiology. At the time of synthesis, the enzymatic hydrolysis of high-molecular N compounds occurs and it participates in various physiological and chemical processes. An increase in N supply will increase different types of N components in various magnitudes.

11. The NO_3^-–N and NH_4^+–N may exhibit different effects on plant nutrition, growth, and crop production for different plant species, and thus plant species may have certain preferences to NH_4^+ and NO_3^-. The preferences are the demand nature of plant species

in relation to the environmental conditions, and the magnitude absorbed and utilized rate of NH_4^+–N and NO_3^-–N by crops mainly depended on crop types, environmental conditions, especially medium pH, and the external concentration of both NH_4^+–N and NO_3^-–N. The preferences have been evaluated mainly by plant biomass or yield production or N uptake amount by the application of NH_4^+–N or NO_3^-–N alone. Based on current results, crop plants may be classified into four types.

a. Preference to NH_4^+–N
Assimilation of NH_4^+–N consumes much less energy than the use of NO_3^-–N does. The preference for NH_4^+ relative to NO_3^- increases strongly with decreasing temperatures, probably reflecting the greater costs of metabolic energy associated with absorption and assimilation of NO_3^- compared to NH_4^+. Sole NH_4^+–N nutrition may cause plant toxicity, rhizosphere acidification, cation uptake reduction and organic acid synthesis decline, and leaf expansion objections. For this reason, only some plants prefer NH_4^+–N. Plant species differ in tolerance to NH_4^+: barley is NH_4^+-sensitive, while rice is NH_4^+-tolerant. In acid or low redox soils, nitrification is inhibited, and NH_4^+ is the predominant form of available N. Adapted to such conditions, plants have a distinct preference for NH_4^+ such as some tree, some grassland species, and some crops. Crop plants containing high amounts of carbohydrates, such as sweet potato, potato, and tea trees, prefer NH_4^+–N. Rice prefers NH_4^+, but application of NH_4^+–N at early stages and NO_3^-–N at late stages can give the best results.

b. Preference to NO_3^-–N
NH_4^+–N and urea fertilizers applied to soils can rapidly get converted into NO_3^-–N, and in most arable soils, NO_3^-–N (6.0 mmol dm^{-3} as an average) is much larger than NH_4^+–N (only 0.77 mmol dm^{-3}). For the majority of plants, NO_3^-–N nutrition is superior to NH_4^+–N nutrition and plants adapted to calcareous, high pH soils, use NO_3^- preferentially. NH_4^+–N can decrease the growth of tomato, maize seedlings, potato, pea, and cucumber. For this reason, many higher plants such as wheat, cotton, sunflower, hemp, sugar beet, tobacco, proso, millet, sorghum, and many vegetables prefer NO_3^-–N, and application of NO_3^-–N at the same N amount as NH_4^+–N has often given a higher yield. With an increase of N concentration in the environment, the superiority of NO_3^-–N increases.

Application of NO_3^-–N as the sole N source or a combination of higher NO_3^-–N to lower NH_4^+–N to wheat gave the best results in increasing crop yield and improvement of crop growth, especially in soils deficient in N supply or when wheat-sowing time was delayed. This is in relation to NO_3^-–N accumulation in plants for improvement of plant N nutrition at later stages. Therefore, the accumulation of NO_3^-–N in plants is a measure of N storage for adaptation to the detrimental environment or conditions plants may encounter.

Vegetables, especially leaf vegetables, strongly prefer NO_3^-–N to NH_4^+–N. In solution culture, the addition of NO_3^-–N in solution as the sole N source ensures good vegetable growth and has no detrimental effect on plant growth, while NH_4^+–N exhibits the detrimental effect on vegetable growth, and even makes plants die. Sugar beet responds better to NO_3^-–N than to NH_4^+–N at high N rates. NO_3^-–N does not only increase sugar beet yield but it also increases sugar content. Tobacco needs NO_3^-–N, and application of NO_3^-–N improves its yield and quality. Although rice prefers NH_4^+–N, supplement of some NO_3^-–N at late growing stages significantly promotes rice growth and increases its yield. Addition of NO_3^- to apple tree seedlings can increase SOD and POD activities. Application of NO_3^- to *V. natans* increased the biomass and total soluble sugar compared to NH_4^+ or urea. The NO_3^- accumulation in plants is mainly caused by excessive N fertilization, and hence, rational N fertilization is the key measure for reduction of NO_3^- accumulation in eatable parts of plants.

c. NH_4^+–N and NO_3^-–N have equal effects
Some results from current and early studies show that NO_3^- and NH_4^+ fertilizers have no different effects on crop yield, plant growth, and N uptake with the exception that the N loss from NO_3^-–N fertilizer was higher than from NH_4^+–N fertilizer.

d. The combinative use of the two N sources is superior to either NH_4^+ or NO_3^- alone.
Combinative use of NH_4^+–N and NO_3^-–N has a much better effect on crop growth and crop yield than the application of either alone by taking each advantage and avoiding each disadvantage in buffering and maintaining the stability of cell and environmental pH, regulating the absorption of other cations and various enzyme activities, rationally using carbon skeletons, and alleviating the NH_3 toxicity. The optimum ratio of NH_4^+–N to NO_3^-–N differs for different species and with plant growing stages and plant age. The optimum ratio is

from 5:5 to 3:7 for spinach, 1:3 for tobacco, 1:1 for tomato, 75:25 and 50:50 for rice, Responses of plants to the two N forms are related to plant growing ages. Tomato prefers NH_4^+–N at early growing stages, while NO_3^-–N at late stages. Maize can rapidly take up more NH_4^+ at the seedling stage than NO_3^-–N, but at late stages, takes up more NO_3^-–N. Rice took up NH_4^+–N dominantly at vegetative growing stages while some NO_3^-–N uptake occurred at the reproductive stage.

e. Enhancement of NH_4^+–N

When media have coexisting NH_4^+–N and NO_3^-–N, many crops have higher dry matter production, N uptake amounts, and N utilization efficiency compared to either alone. Based on this fact, a concept, enhancement of NH_4^+–N, that is, increasing the concentration of NH_4^+–N in the soil under the condition of NO_3^-–N being predominant, has been proposed. Enhancement of NH_4^+–N nutrition significantly increased chlorophyll contents, photosynthetic velocity, NRA, cumulative N amount, dry matter production, and yield.

f. Enhancement of NO_3^-–N nutrition to rice

Rice is actually nourished by NH_4^+–N and NO_3^-–N together. The ENNN to rice increases the total root biomass, especially the lateral roots, increases rice Vmax, NH_4^+-transport protein expression, the total N uptake, utilization of NH_4^+–N, and crop yield compared to NH_4^+ nutrition alone. The promotion of root growth by NO_3^- has attributed to the regulation of carbohydrates, promotion of the rapid synthesis, and transport of root endogenous hormones. Only when NO_3^- exist, does the GS–GOGAT system in rice root proplastid work actively, and make the absorbed NH_4^+ to be rapidly assimilated and used by plants, and then further enhances N uptake and transport to shoots.

12. Reasons for the preference of crop plants to the two N forms

The plant preferences to NH_4^+–N and NO_3^-–N are determined by many factors. Plant species, cultivars, and their growing stages are internal factors in relation to plant properties. Most plants have evolved under conditions of a very low NH_4^+ supply with NO_3^-–N being the predominant form of available N. These plants are not adapted to NH_4^+ as their major or sole N source. Other plants developed under NH_4^+-fed conditions are capable of using NH_4^+ as the N source, and lack of NO_3^- reductive ability may make some of these plant species impossible to efficiently use NO_3^- as their major source of N. In addition, some plant species may have significant NRA in their roots but very low amounts in their leaves.

When the root NR system becomes saturated with NO_3^-–N, the leaves cannot act as the major sink for the assimilation of the surplus NO_3^-; hence, these plants also cannot efficiently use NO_3^- as the major source of N. The carbohydrate content in plants has a great influence on the preference; shortage of sugar content can inhibit the reduction of NO_3^-–N and the synthesis of amides, and thus affect plants' NO_3^- preference.

The preferences are also determined by the two ions' (NH_4^+ and NO_3^-) chemical properties that affect N loss and N efficiency to plants and the uptake of other anions and cations. NO_3^- nutrition promotes cation accumulation to high levels, and this in turn results in the production of organic anions for balancing cations and anions in the plant. High concentrations of the organic acid anions may bind with Fe in both the roots and leaves, thereby interfering with the functions of Fe in the plants. Although ambient NH_4^+ inhibits the plant uptake of Mn and Al, the uptake of NH_4^+ can decrease soil pH that may in turn increase the solubility of Mn and Al, and therefore NH_4^+ nutrition may, to some extent, be involved in the tolerance of plants to high levels of Al and/or Mn.

External or environmental factors such as media pH, cations and anions, NH_3 concentration in solution, temperature, illumination, and aeration also have great effects on the preferences.

13. Plants can tolerate high levels of soil NO_3^-, and the concentration of NO_3^- may increase to several percents in the plant before phytotoxicity apparently occurs. The accumulation of large amounts of NO_3^- in plant tissues may increase anion uptake than cation uptake and thus upset the cation–anion balance and osmoregulation. An excess supply of NO_3^- might be toxic to plants, but the opportunity is much less than in the case of NH_4^+–N.

The toxicity of NH_4^+/NH_3, as the sole N source, is characterized by an immediate restriction in plant growth, stem lesions, leaf area decline, total biomass reduction, and finally plant death. Influence of root growth and production of short, thick, less branched, and darkly colored roots is the particular feature of the toxicity.

Different species may have different tolerances to the NH_4^+ or NH_3, and thus, the serious magnitude of the toxicity can appear to be different among species and even among cultivars in the same species. Depending on the plant species and particular growth conditions, each of these factors may contribute to the toxicity.

14. Two hypotheses have been put forward to explain the physiological cause of the toxicity. Some scientists consider that the toxicity is due mainly to free NH_3 that affects plant growth and metabolism at low concentration levels at which NH_4^+ is found to be not harmful. Different from NH_4^+ ions, NH_3 molecules can directly penetrate the membrane and enter cells through diffusion, resulting in cytoplasmic pH increase that inhibits GS activity as the primary cause. The initially toxic concentration of water soluble NH_3 is 0.15 mmol L^{-1}, and the lethal concentration is 6.0 mmol L^{-1}. The NH_3 toxicity is affected by medium pH and NH_4^+ concentration. However, based on the fact that under conditions without a possibility to produce NH_3, or the possibility being removed, the toxicity effect on inhibition of plant growth still exists, and the toxicity is often strengthened by water stress, the NH_4^+ toxicity is proposed, which, as a whole, decreases the uptake of essential cations, induces disorders of NH_4^+-induced pH changes, or produces excessive consumption of sugars; and, from the physiological point, injures membrane and cell wall structure as well as membrane enzyme activity as the primary cause and changes the enzyme activities and metabolism, retards nutrient absorption and plant growth as the secondary cause. Water stress has an unfavorable effect on membrane stability and under water-stressed condition, NH_4^+ may intensify the ill effect, make ATPase in membranes difficult to display its role in pumping H^+, result in cell H^+ accumulation and changes of some related enzyme activities, induce or promote the dissociation of Ca^{2+} in membranes and make the membrane function difficult to be maintained, induce or strengthen some ion leakages out of roots, and bring about nutrient loss while NO_3^- nutrition may have a significant role in alleviating the NH_4^+ toxicity. The toxicity of NH_4^+/NH_3 to plants is the combinative result of internal and external factors. As an internal factor, carbohydrate amounts in plants do not only affect N metabolism but also the toxicity. Supply of α-ketoglutarate to higher plants tissues can greatly reduce internal NH_4^+ concentration and thus alleviate NH_4^+ toxicity. Plants need a very high level of carbohydrates and carbohydrate-degrading reactions provide the necessary substances for amide formation during NH_4^+ assimilation. Strong acidification of rhizosphere soils is an important environmental factor, and the medium acidification associated with NH_4^+ absorption has been shown to be toxic to many crop plants The degree of the toxicity is associated with crop sensitivity to pH.

15. Six crops growing in solution culture with equal NH_4^+–N and NO_3^-–N in entire life cycles show that crops did not absorb equal amounts of each N form, varying not only with crop species, but also with growing stages of each crop and solution pH. The media pH affects wheat relative uptake amount of NH_4^+–N and NO_3^-–N. The uptake ratio of NH_4^+–N to NO_3^-–N of wheat varies with initial pH and growth stages. Caused by the unequal uptake of the two N forms, the solution pH for wheat culture was decreased with a higher NH_4^+–N uptake while it increased with a higher NO_3^- uptake. The increasing solution pH for all pH treatments had a trend toward neutralization. The N absorption curves (NH_4^+–N + NO_3^-–N) of wheat are similar under different pH conditions, but the amounts of NH_4^+–N and NO_3^-–N are greatly different. As a whole, at an initial pH of 6.5, the total uptake amount of the two N forms is the largest, and at pH 5.0 and 8.0, there is no significant difference in the total uptake amount. Rice exhibits different responses to NH_4^+–N and NO_3^-–N at different stages. The amounts and ratios of NH_4^+–N to NO_3^-–N taken up by rice vary with its growth stages. Application of NH_4^+–N at early stages and alternatively applying NH_4^+–N and NO_3^-–N at late stages may promote rice growth and raise its yield. The amounts of NH_4^+–N and NO_3^-–N absorbed by rice are closely related to their Vmax.

16. Effect of NH_4^+–N and NO_3^-–N forms on some morphological and physiological characteristics of crop plants.

a. Effect of N forms on crop root morphology
Supply of NO_3^-–N can increase lateral root growth, but supply of NH_4^+–N inhibits root growth and produces abnormal growth of lateral roots.

b. Effect of N forms on chlorophyll contents and photosynthesis
For coexistence of NH_4^+–N and NO_3^-–N, the chlorophyll contents and the net photosynthetic rate are the highest, followed by NO_3^-–N, while NH_4^+–N is the lowest. NH_4^+–N, NO_3^-–N, and their mixed N source exerted a greater effect on mesophyll conductance: the highest occurs for the mixed N source, followed by NO_3^-–N, and the smallest is for NH_4^+–N

c. Effect of N forms on N-containing compounds
Some results show that that with the supply of NH_4^+–N, the total N, free amino acids, and amides are higher than NO_3^-–N, but others show that the two N forms have no great influence on crop N accumulation and N-containing components.

d. Effect of N on carbohydrate and nutrient accumulation
Application of NO_3^-–N exerts a higher effect on carbon assimilation than NH_4^+–N does, and benefits the accumulation of sucrose while NH_4^+–N benefits starch accumulation. Full supply of NO_3^-–N can increase plant organic acids, while full supply of NH_4^+–N can consume large amounts of organic acids and carbohydrates and reduce the sugar content. Addition of $NO^-{}_3$–N alone can increase the total soluble sugar, reductive sugar and structured polysaccharide contents in roots, stems, and leaves of maize seedlings. The two N forms also affect the mineral ion uptake and nutrient accumulation in plants.

e. Effect of N forms on NRA
The amount and activity of NR, as an induced enzyme, are closely linked to NO_3^-–N. In a certain range, the more the NO_3^-–N is taken up, the higher the NRA will be, and to a certain extent, the NRA is positively correlated to NO_3^-–N accumulation in plants. The use of NH_4^+–N as the sole N source can inhibit NRA and some organic N may have different effects. Among various factors that affect NRA, NO_3^-–N, the substrate, is most significant.

f. Effect of N forms on turgor pressure
NO_3^-–N increases or maintains whereas NH_4^+–N significantly depresses the leaf water potential and turgor pressure as shown in some crops.

g. Effect of N forms on protective enzymes
Different N forms may unavoidably produce different effects on the ROS levels, antioxidant enzyme activity, and the protective enzyme activities. With a mixed supply of NH_4^+–N and NO_3^-–N, the activities of SOD and CAT are the highest in leaf blades. NO_3^- addition can promote the survival and restoration of plants that were damaged by waterlogging and can produce NO that in a suitable amount can increase SOD and CAT activities under salt stress, decrease the formation rate and cumulative amounts of H_2O_2 and $O_2{\cdot}^-$ as well as MDA, and increase in antioxidant substances.

h. Effect of N forms on hormones
NH_4^+–N and NO_3^-–N can affect the formation and activity of hormones to inhibit or promote cell elongation and therefore plant growth. Compared to NH_4^+–N, the application of NO_5^-–N significantly promotes the rapid synthesis and transport and activity of root-originated hormone CTK and auxin IAA. The IAA content of

tobacco seedlings grown in 100% NO_3^-–N solution was larger than those grown in 50% NH_4^+–N + 50% NO_3^-–N solution. The auxin concentration in the aboveground part of plants cultured by NO_3–N was higher than that by NH_4–N. The NO_3^-–N may influence lateral root growth though the regulation of auxin synthesis and transport. For the supply of NH_4^+–N to plants, the transport of CTK from the roots to the shoots is reduced, and the shoot growth of some crops is rapidly inhibited.

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CHAPTER SIX

Flaming as an Alternative Weed Control Method for Conventional and Organic Agronomic Crop Production Systems: A Review

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Contents

Abstract

The interest for organic crop production is in the increase due to a strong demand for organic food from consumers and an attractive income potential for farmers. Weeds pose one of the major problems in crop production and are responsible for significant crop yield reduction. The problem of controlling weeds without synthetic herbicides under the rules of organic agriculture is challenging. The increase in the number of herbicide-resistant

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weeds, the increase in herbicide cost, and the movement of herbicides into surface and ground water have sparked public awareness and restrictions on herbicide use. For these reasons, weed scientists are considering alternative and integrated weed management practices to reduce herbicide inputs and impacts. The use of propane for flame weeding can be adopted as one of the alternatives to chemical weed control, as it eliminates concerns over direct residual effects on soil, water, and food quality and can lessen the reliance on herbicides, hand weeding, and/or mechanical cultivation. Flame weeding is an acceptable weed control option in both organic and conventional production systems. A greater knowledge on the development of dose–response curves for determining the appropriate propane dose for effective weed control in major agronomic crops is needed to improve flame-weeding strategies. The dose–response curves for weeds and crops are important so that the lowest effective dose of propane can be applied for weed control in agronomic crops, which saves energy and reduces production costs. Depending on the desired level of weed control or tolerable crop injury level, a propane dose could be selected to either control the weed, or reduce its competitive ability against the crop. In this chapter, we will provide an overview of the findings from the flaming research that has been conducted for the last six years at the University of Nebraska, USA, or reported in pertinent newest literature. This chapter will improve our existing knowledge about flame weeding and will present better general guidelines for both organic and conventional crop producers interested in flaming techniques for weed control.

1. INTRODUCTION

Integrated weed management (IWM) has been defined as a multidisciplinary approach to weed control, using the application of numerous alternative control measures (Knezevic et al., 1994; Swanton and Weise, 1991). IWM involves a combination of cultural, mechanical, biological, genetic, and chemical methods for effective and economical weed control that reduces weed interference with the crop while maintaining acceptable crop yields (Knezevic et al., 2002; Swanton and Murphy, 1996). None of the individual control measures on their own can be expected to provide complete weed control. However, if the various components of IWM are implemented in a systematic manner, significant advances in weed control technology can be achieved (Swanton and Weise, 1991). An IWM approach advocates the use of all available weed control options that include (i) selection of a well-adapted crop variety or hybrid with good early season vigor and appropriate disease and pest resistance (plant breeding); (ii) appropriate planting patterns and optimal plant density, improved timing, placement, and amount of nutrient application; (iii) appropriate crop rotation, tillage practices, and cover crops; (iv) suitable choice of mechanical, biological, and chemical weed control methods; and (v) alternative weed control tools (flaming, steaming, infrared radiation, sand blasting, etc.).

Most of the North American agronomic cropping systems are based on the genetically modified crops (e.g. glyphosate-, glufosinate-, or imidazolinone-resistant crops; Elezovic et al., 2012). These crop production systems rely heavily on the use of postemergence herbicides. Repeated use and relying on herbicides resulted in an increasing number of herbicide-resistant weeds, shifts in weed species population, higher cost of chemical control measures, leaching of herbicide into ground water, and surface water as well as herbicide residues in drinking water and food, which have sparked public awareness and restrictions on herbicide use (Geier et al., 2006; Knezevic et al., 2009a; Rifai et al., 2002). Herbicides have often been cited as one of the main factors responsible for causing a general impoverishment of the flora and fauna in the agricultural landscape (Marshall et al., 2003; Melander et al., 2005). To address these challenges, many countries have developed policies that mandate the reduction of herbicide use and provide incentives to producers for reducing overall chemical use (Kristoffersen et al., 2008; Rask et al., 2012a, 2012b). Using the principles of IWM can aid in reducing the dependence on herbicides. For these reasons, weed scientists worldwide are studying alternative weed management practices to reduce herbicide inputs and impacts (Rifai et al., 2002). There is currently a renewed interest in flame weeding as an alternative to other methods of weed control, including chemicals, especially due to the latest advances in the flame engineering technology (Bruening, 2009; Bruening et al., 2009; Knezevic and Ulloa, 2007; Wszelaki et al., 2007).

Propane flaming is a process of exposing plant tissues to the heat coming from a propane burner. Propane-fueled flame weeding is an acceptable weed control option in organic production and has received renewed interest for both organic and conventional cropping systems (Bond and Grundy, 2001). Flame weeding can be also used in other situations in which the presence of herbicide is undesirable, such as in cities, parks, and other urban settings (Andreasen et al., 1999; Hansen et al., 2004; Rask et al., 2012b). Flaming should not be confused with burning as plant biomass does not ignite, but heats them rapidly to the point of rupturing cell membranes and tissues, which result in weed death (Lague et al., 2001).

Organic crop production in the USA has achieved an increasing awareness and acceptance by producers over the last decade (Derksen et al., 2002). The main reasons for the adoption of organic farming practices are the detrimental effects of synthetic chemicals, heightened public concern about chemical residues in food resulting in a strong demand for organic products and attractive organic price premiums for farmers

(Abouziena et al., 2009; Penfold et al., 1995). Sales of organic products have grown to >20% each year for the past decade, which makes the organic industry the fastest growing segment of the US agriculture. Despite the growth, current certified organic land remained only at about 0.72% of the total US farmland production (Greene, 2009). In the USA, the area under certified organic cropland increased >400% from 1995 to 2008, as the total organic cropland grew from 260,000 ha in 1995 to over 1 million ha in 2008 (Greene, 2009). Wheat (*Triticum aestivum* L.) was the largest organic grain/seed crop with the production area of about 170,000 ha followed by organic maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.], which were raised to nearly 79,000 ha and 51,000 ha, respectively, in 2008 (Greene, 2009). High remunerative prices and reduced cost of inputs relative to vegetable crops have motivated growers to increase the area under agronomic crop production.

Weeds pose one of the major problems in both conventional and organic crop production systems and are responsible for significant crop yield reduction (Stopes and Millington, 1991). Lack of effective weed management practices is widely reported to be a major production-related problem in organic crops (Gianessi and Reigner, 2007; Hiltbrunner et al., 2007; Wszelaki et al., 2007). However, controlling weeds in organic farming is challenging and requires the use of many techniques and strategies to achieve economically acceptable weed control and crop yields.

The problem of controlling weeds without synthetic herbicides under the rules of organic agriculture is challenging (Kruidhof et al., 2008). There are very few herbicides approved for use in organic production, but they are costly and nonselective and thus can injure crops (Knezevic, 2009). Therefore, organic producers rely extensively on mechanical cultivation and hand weeding for their weed control (Hiltbrunner et al., 2007). However, repeated cultivation can accelerate loss of soil organic matter, destroys soil aggregate, increases the chance for soil erosion and promotes emergence of new weed flushes (Cloutier et al., 2007; Hiltbrunner et al., 2007; Wszelaki et al., 2007). The labor required for hand weeding is expensive (e.g. ranging from \$300 to \$800 ha^{-1}), time consuming, and difficult to organize (Kruidhof et al., 2008). Hence, system-oriented approaches to weed management that make better use of alternative weed management tactics need to be developed (Kruidhof et al., 2008). Propane flaming is one of the most promising alternatives for weed control in organic cropping systems, and with the potential for use on the conventional crops as well (Ulloa et al., 2010a, 2010b, 2010c, 2010d, 2010e, 2010f; 2011a, 2011b, 2012).

1.1. Rationale

Flaming controls weeds primarily by rupturing the cell membranes that leads to subsequent tissue desiccation (Lague et al., 2001). Propane burners can generate combustion temperatures of up to 1900 °C, which raises the temperature of the exposed plant tissues rapidly (Ascard, 1998). An increase of temperature to >50 °C inside the plant cells can result in the coagulation (denaturation and aggregation) of membrane proteins leading to loss of membrane integrity (Lague et al., 2001; Parish, 1990; Pelletier et al., 1995; Rifai et al., 1996). Consequently, flamed weeds die, or their competitive ability against the crop is severely reduced. The susceptibility of plants to flaming largely depends on their heat avoidance, heat tolerance, or both (Ascard, 1995). The extent to which heat from the flames penetrates plants depends on the flaming technique and leaf surface moisture (Parish, 1990).

The effects of flaming on plants are influenced by several factors including temperature, exposure time, and energy input (Ascard et al., 2007). Depending on the exposure time, protein denaturation may start at 45 °C (Ascard et al., 2007). Temperatures in the range of 95–100 °C have been reported to be lethal for leaves and stems and should be applied for at least 0.1 s (Ascard et al., 2007).

Heat from the flames has a direct effect on the cell membranes and an indirect effect during the subsequent tissue desiccation. Cellular death after flaming treatment is primarily due to the initial thermal disruption of cellular membranes rapidly followed by dehydration of the affected tissue. Tissue dehydration occurs mainly due to the expansion of the cell contents (made up to 95% water), subsequent bursting of the cell membranes, and coagulation of membrane proteins (Bond and Grundy, 2001; Leroux et al., 2001).

The efficacy of flame weeding was reported to be influenced by several factors, including the growth stages of the plant (Ascard, 1994, 1995; Ulloa et al., 2010a, 2010b, 2010c, 2010d, 2010e, 2010f), the physical location of the growing point at the time of flaming (Ascard, 1995; Hansson and Ascard, 2002; Knezevic and Ulloa, 2007), the presence of protective layers of hair or wax, and lignification (Ascard, 1995; Ascard et al., 2007), the technique of flaming (Parish, 1990), the regrowth potential of plant species (Ascard, 1995; Ascard et al., 2007), and the leaf relative water content of plant species (Ulloa et al., 2012).

1.2. Objective

The objective of this chapter is to provide the newest information on weed and crop tolerance to flaming. The effects of broadcast flaming treatments with different propane doses will be discussed in the context of weed control and crop tolerance. To optimize the use of propane flaming as a weed

control tool, the biologically effective dose (ED) of propane for control of major weed species and tolerance of major crops must be determined (Knezevic and Ulloa, 2007). These dose–response curves for weeds and crops are important so that the lowest ED of propane can be applied for weed control, which saves energy and results in lower production costs. Depending on the desired level of weed control or tolerable crop injury level, a propane dose could be selected to either control the weed or reduce its competitive ability against the crop.

Previous research has demonstrated that weed susceptibility to flaming varied among species and plant size (Ascard, 1994, 1995; Cisneros and Zandstra, 2008; Domingues et al., 2008; Sivesind et al., 2009). Ascard (1994) reported that growth stage of weeds at the time of flaming determines plant sensitivity to heat, with small weeds being more sensitive to flaming than large ones. Several earlier flaming studies in vegetable crops had demonstrated that this method could be used as an alternative to control weeds (Ascard, 1995; Wszelaki et al., 2007). However, little information is available on the development of dose–response curves to determine the correct propane dose for controlling weed species without affecting crop yields. Therefore, we will present an overview of the findings from the flame-weeding research that has been conducted for the last six years at the Haskell Agricultural Laboratory of the University of Nebraska, Concord, Nebraska, USA. The newest information on flame weeding from the literature is also included to improve the overall knowledge about flame weeding and provide better guidelines to organic and conventional crop producers interested in flaming as a tool for weed control.

1.3. Flaming Specifications and Equipment

The flaming treatments were conducted at different growth stages of each weed species and crop, and the growth stages were based on the number of leaves. Flaming treatments were applied using a custom-built research flamer mounted on a four-wheeler (all-terrain vehicle), which was driven either perpendicularly across the planted weed rows, or parallel to the crop rows in separate studies. The flamer used propane as a source for combustion, and there were four burners "LT 2 × 8 Liquid Torch" (Flame Engineering, 2007) mounted either 30 cm apart, which allowed to flame a 120-cm-wide band across the weed rows, or 76 cm apart to position flames directly over the crop rows. Burners were mounted on the back of the four-wheeler at 20 cm above the soil surface and angled back at 30°. Flaming treatments were carried out at a constant speed of 6.4 km h^{-1}, and propane pressure was

changed to deliver an appropriate propane dose. The calibration procedure was based on combining propane pressure and operating speed (Knezevic et al., 2007). The above setup provided either a 120-cm-wide flaming swath to flame the weed rows or a 25-cm-wide open flames over each of the four crop rows to purposely cause crop injury; thus, the tolerance of weed/crop to broadcast flaming could be tested.

1.4. Determination of Flaming Efficacy

Efficacy of flaming treatment can be easily determined at the field level by conducting a simple 'fingerprint test.' Immediately after flaming, place a leaf between the thumb and index finger, and firmly press on it. If a darkened impression (fingerprint) is visible on the leaf surface, it is likely the evidence of a loss of internal pressure due to cell water leakage. Plant death occurs as a result of lost water through the broken cell walls and dehydration.



2. RESPONSE OF SELECTED WEED SPECIES TO FLAMING

Response of weed species to broadcast flaming varied among weed species, their growth stages, and propane dose (Leroux et al., 1995; Ulloa et al., 2010a, 2010b). The success of flame weeding depends on flaming weeds at their appropriate growth stage with the proper dose of propane.

2.1. Role of Plant Size on Weed Species Tolerance to Flaming

Smaller weeds (2–8 cm tall, 1–6 leaves) are much easier to control with propane flaming than larger ones (8–50 cm tall, 7–20+ leaves). Plant size at the time of flaming had a significant effect on plant sensitivity to heat, with small plants being more sensitive than large ones. Others also reported that flaming is more effective on most weeds at an early growth stage (Ascard, 1995, 1998; Cisneros and Zandstra, 2008; Domingues et al., 2008; Knezevic et al., 2009d; Sivesind et al., 2009). The thin and delicate plant tissues at the early vegetative stage are very heat sensitive, and the plants cannot regrow when they get damaged (Ascard, 1994). The higher thermal sensitivity of younger plants is mainly due to the fact that they have thinner leaves, lower shoot biomass, and less protected meristems (Ascard, 1995). Another critical part of the young plants is the growing point in the shoot apex, which also determines the heat tolerance of plants (Ascard, 1994). In older plants, the shoot apex is often protected by the surrounding leaves, and the larger amount of reserve food in the roots also gives an increased capacity for

regrowth (Ascard, 1995). The tolerance of different plant parts to flaming can also be influenced by other factors, including the presence of protective layers of hair and/or wax, lignification level, and condition of the overall plant water status (Ascard, 1995). About 90% control of *Abutilon theophrasti* Medik. can be obtained with a single dose of 42, 56, and 102 kg of propane ha^{-1} for the 5-leaf, 7-leaf, and 16-leaf stages, respectively (Ulloa et al., 2010b, Fig. 6.2, Table 6.1). About 134 kg ha^{-1} of propane was needed to obtain 90% control in *Setaria pumila* (Poir.) Roemer and J.A. Schultes at the flowering stage compared with significantly lower doses of 72 and 86 kg ha^{-1} of propane for the 2-leaf and 4-leaf stages, respectively, suggesting that weeds tolerated more heat with the increase in plant size (Ulloa et al., 2010a, Fig. 6.1, Table 6.1). Other broadleaf and grass weeds exhibited a similar response to propane doses (Table 6.1). Dose–response studies conducted by Ascard (1994, 1995) and Ascard (1998) showed that a single dose of 10–40 kg of propane ha^{-1} was required to achieve 95% control of sensitive species, such as *Chenopodium album* L. with 0- to 4-leaves, whereas plants with 4- to 12-leaves required 40–150 kg propane ha^{-1}. These results suggest that flaming is the most effective on most weed species at an early growth stage.

2.2. Role of Plant Type on Weed Species Tolerance to Flaming

Grasses are difficult to control with flaming than are broadleaf species (Ulloa et al., 2010a, 2010b, Figs 6.1 and 6.2, Table 6.1). Such differences are likely a result of the physical position of the growing point at the time of flaming (Ascard, 1995; Knezevic and Ulloa, 2007; Knezevic et al., 2009b, 2009c, 2009d). The growing point in grass species during early growth stages is below the soil surface and is thus protected from the flame. In contrast, the growing point in broadleaf species is above the ground and is thus exposed to the flame.

Broadleaf weeds, such as *Amaranthus rudis* Sauer, *Amaranthus retroflexus* L., *Convolvulus arvensis* L., *Kochia scoparia* (L.) Schrad., *Ipomoea hederacea* Jacq., *A. theophrasti* Medik., *Hibiscus trionum* L., and *Ambrosia artemisiifolia* L., can be effectively controlled (90%) with a single dose of about 16–70 kg of propane ha^{-1} when flamed at early growth stages ranging from 2-leaf to 14-leaf (Figs 6.1 and 6.2, Table 6.1). A 90% control of grass type weeds such as *Setaria viridis* (L.) Beauv., *S. pumila* (Poir.) Roemer and J.A. Schultes, and *Echinochloa crus-galli* (L.) Beauv. can be achieved with a propane dose of about 47–90 kg ha^{-1} when flamed at their early growth stages (2–7 leaves; Figs 6.1 and 6.2, Table 6.1). An 80% control of these grass weed species can be obtained with a propane dose ranging from 34 to 58 kg ha^{-1} when flamed at their early growth stages (2–7 leaves). It should be noted that the most

Table 6.1 List of Broadleaf and Grass Weed Species, Their Growth Stages with Corresponding Heights, and Doses of Propane (kg ha^{-1}) Needed to Obtain 80% (ED_{80}) and 90% (ED_{90}) Weed Control

Weed species	Growth stage	Plant height (cm)	Propane dose (kg ha^{-1})	
			ED_{80} (±SE)	ED_{90} (±SE)
A. rudis Sauer	3-Leaf	3	29 (4)	40 (7)
	5-Leaf	10	36 (6)	46 (9)
	Flowering	38	33 (3)	46 (6)
A. retroflexus L.	3-Leaf	6	47 (7)	70 (14)
	5-Leaf	9	48 (5)	68 (8)
	Flowering	46	59 (3)	86 (6)
C. arvensis L.	8-Leaf	4	28 (3)	40 (6)
	10-Leaf	11	22 (2)	31 (5)
	40-Leaf	32	32 (4)	57 (10)
K. scoparia (L.) Schrad.	6-Leaf	2	37 (5)	49 (9)
	10-Leaf	8	26 (5)	37 (11)
	Flowering	45	46 (8)	76 (20)
I. hederacea Jacq.	10-Leaf	7	39 (4)	55 (9)
	14-Leaf	10	47 (3)	61 (6)
	Flowering	40	45 (6)	66 (13)
A. theophrasti Medik.	5-Leaf	6	28 (4)	42 (8)
	7-Leaf	18	37 (4)	56 (8)
	16-Leaf	98	56 (9)	102 (25)
H. trionum L.	3-Leaf	3	32 (4)	53 (9)
	5-Leaf	8	34 (4)	51 (8)
	Flowering	50	39 (5)	72 (14)
A. artemisiifolia L.	4-Leaf	3	14 (1)	16 (1)
	14-Leaf	20	37 (6)	119 (16)
	26-Leaf	78	61 (6)	108 (12)
S. viridis (L.) Beauv.	5-Leaf	5	58 (17)	85 (33)
	7-Leaf	13	56 (6)	90 (13)
	Flowering	66	104 (6)	140 (11)
S. pumila (Poir.) Roemer & J.A. Schultes	2-Leaf	4	41 (9)	72 (22)
	4-Leaf	8	52 (11)	86 (24)
	Flowering	49	79 (7)	134 (17)

Continued

Table 6.1 List of Broadleaf and Grass Weed Species, Their Growth Stages with Corresponding Heights, and Doses of Propane (kg ha^{-1}) Needed to Obtain 80% (ED_{80}) and 90% (ED_{90}) Weed Control—cont'd

Weed species	Growth stage	Plant height (cm)	Propane dose (kg ha^{-1})	
			ED_{80} (±SE)	ED_{90} (±SE)
E. crus-galli (L.) Beauv.	4-Leaf	6	34 (6)	47 (12)
	7-Leaf	18	45 (5)	76 (11)
	Flowering	83	78 (18)	128 (49)

Ulloa et al. (2010a, 2010b).

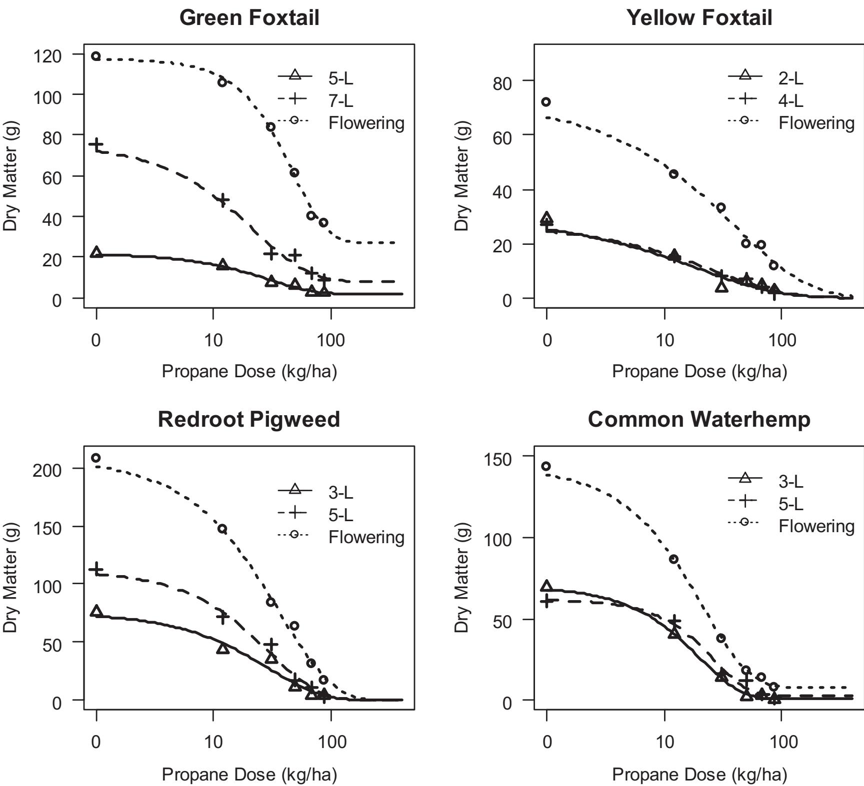


Figure 6.1 Effects of propane dose on dry matter reduction of green foxtail [*S. viridis* (L.) Beauv.], yellow foxtail [*S. pumila* (Poir.) Roemer and J.A. Schultes], redroot pigweed (*A. retroflexus* L.), and common waterhemp (*A. rudis* Sauer) as influenced by growth stage. *(Ulloa et al. (2010a))*.

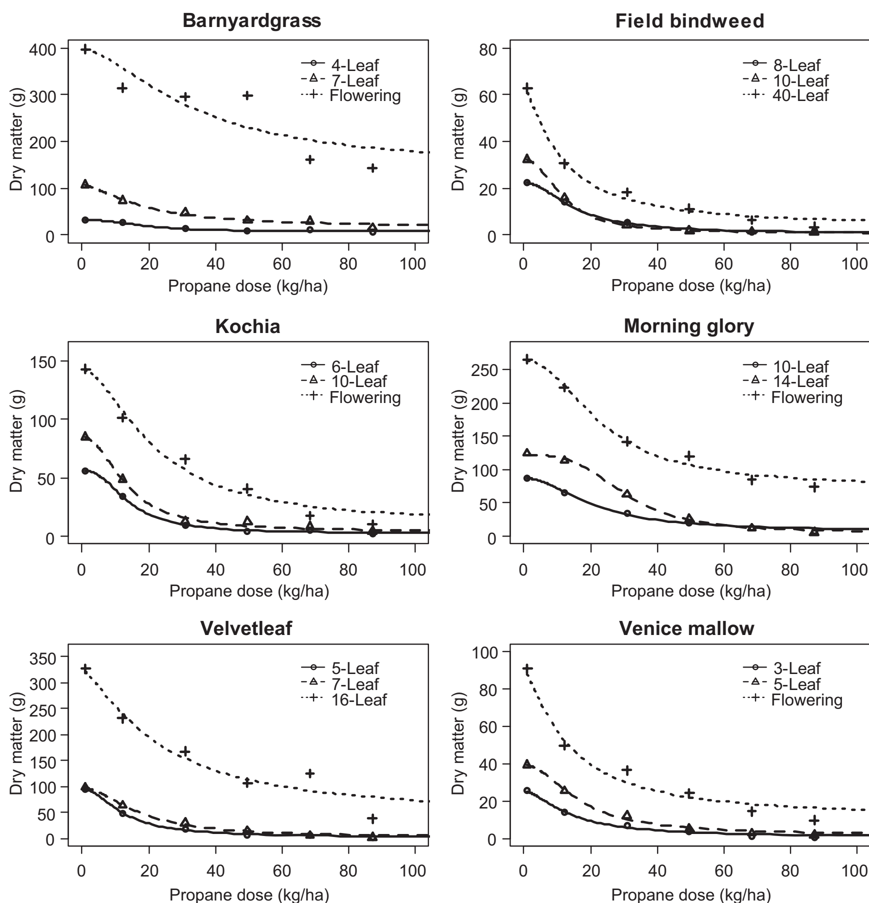


Figure 6.2 Effects of propane dose on dry matter reduction of barnyardgrass [*E. crus-galli* (L.) Beauv.], bindweed (*C. arvensis* L.), kochia [*K. scoparia* (L.) Schrad.], morning glory (*I. hederacea* Jacq.), velvetleaf (*A. theophrasti* Medik.), and Venice mallow (*H. trionum* L.) as influenced by growth stage. (*Ulloa et al. (2010b)*).

organic weed control methods are not as efficient as chemical control; thus, an 80% weed control in organic farming is considered as an acceptable level of control.

The annual broadleaf and grass weed species responded differently to the flame and heat (Ulloa et al., 2010a, 2010b). Leaves of annual broadleaf species were completely desiccated within a few days after flaming, and there was no plant regrowth especially when flamed with doses >60 kg of propane ha^{-1}. Leaves of grass species turned white shortly after flaming, leaving an appearance of a dead plant; however, within a week or two, plants

begin to recover with the growth of new leaves. Control of grass species can be improved by second flaming operation within 7–10 days after the first flaming. If an additional flaming is not feasible, an aggressive cultivation to push soil onto the top of the flamed grass is very helpful in preventing the regrowth of grass. This differential tolerance of grass and broadleaf species to broadcast flaming is consistent with previous findings of Ascard (1995) who reported that grass species flamed at early growth stages showed initial plant stunting followed by plant recovery after a few weeks. Similar responses were also reported for field crops belonging to grass versus broadleaf families. Teixeira et al. (2008) demonstrated that soybean flamed at the third trifoliate stage had higher injuries than maize flamed at the 5-leaf stage, which regrew a week later.

A propane dose of 60 kg ha^{-1} if properly used is highly effective in controlling many broadleaf weeds at early growth stages (up to 20 cm tall), providing >90% control of most common broadleaf weeds commonly found in agronomic crops of the Midwestern USA (Ulloa et al., 2010a, 2010b). The same dose of propane can provide about 80% control of several grass species when flamed at their vegetative growth stages (2–7 leaves; Ulloa et al., 2010a, 2010b).

Flaming has no residual soil activity, and the heat cannot penetrate deeper into the soil profile; therefore, flaming does not provide control of root structures of perennial weeds. However, biannual and perennial weeds such as *Cirsium arvense* (L.) Scop., *Carduus crispus* L., and *Carduus acanthoides* L. are extremely sensitive to heat, and their above-ground tissue and biomass can be easily desiccated. A propane dose of 15 kg ha^{-1} turned leaves of *C. arvense* (L.) Scop. completely black within 24 hours after flaming; however, the plants regrew from their below-ground meristems within a few weeks. Flaming must be repeated several times during the season to control these weeds (Ascard, 1995). It is important to mention that the most important factor affecting the success of thermal weeding is the life cycle of the weeds, and overall perennial species are more tolerant to flaming than annual species (Rifai et al., 2002). In another study, Rifai et al. (1999) also reported <60% reduction in perennial weed numbers after one, two, or three treatments at a gas dose of 35, 23, or 17 kg ha^{-1} and only 75% after total gas doses of 320 and 216 kg ha^{-1} over four flaming treatments. Repeated flaming over several years can deplete the nutrient reserves from the root and result in completely killing the plant. For example, experiments conducted by Hakansson (2003) and Rask and Andreasen (2007) showed that, to affect perennial plants sufficiently, the second and subsequent treatment should be

carried out after an initial regrowth, but before regrowing shoots become too large.

Environmental factors can also impact flaming. Any kind of moisture, including heavy dew and rain droplets on the leaves, will reduce efficacy of flaming. The presence of moisture on the plant tissue can reduce the amount of heat that can reach inside the plant tissue, as the portion of the heat is wasted on evaporating the surface water present on the leaf. However, the presence of moisture can be useful for (i) reducing the crop injury level and (ii) wetting the crop residue present on the field, thus reducing the chance for fire hazards. Slight irrigation or sprinkle of the crop residue an hour before flaming can also help reduce potential for fire hazards. Irrigation is also useful in promoting early emergence of weed species, which can be controlled with the nonselective broadcast flaming operation. Organic vegetable producers often irrigate prepared seedbeds to stimulate weed germination, and then use flaming to kill weed seedlings before crop planting, or after planting but before crop emergence. Balsari et al. (1994) reported that a single flaming four days after irrigation and one day before transplanting lettuce (*Lactuca sativa* L.) seedlings reduced weed densities by 62%. Flaming before crop emergence has been the predominant thermal weed control method in slow-germination vegetable crops such as onion (*Allium cepa* L.), leek (*Allium porrum* L.), and carrot (*Daucus carota* L.).



3. RESPONSE OF SELECTED AGRONOMIC CROPS TO FLAMING

Knezevic et al. (2009b) and Ulloa et al. (2010c, 2010d, 2010e, 2010f, 2011a, 2011b) conducted a series of studies in which the authors intentionally flamed several agronomic crops such as field maize, pop maize (*Z. mays* L. var. *everta*), sweet maize (*Z. mays* L. var. *rugosa*), winter wheat, grain sorghum [*Sorghum bicolor* (L.) Moench], and soybean under weed-free conditions to cause injuries by positioning torches directly over the crop row. The objective behind such an approach was to determine crop tolerance to flame, and record most likely the worst-case scenario of crop injuries by direct flames and intense heat. The authors pointed out that the tolerance of those agronomic crops to broadcast flaming was dependent on crop species, their growth stages, and propane dose. The crop growth stage is critical in determining whether or not to use a flamer for weed control after crop emergence (Knezevic and Ulloa, 2007). Every agronomic crop is sensitive to heat, and flaming crop at the wrong growth stage could result in severe

yield losses. The key is to position torches (heat source) in such a way that the growing point of the crop is protected during flaming operations. The heat should be directed away from the growing point of the crop to avoid potential crop injury.

3.1. Tolerance of Maize Types to Flaming

Response to broadcast flaming varied among maize types, their growth stages, and propane dose (Knezevic et al., 2009c). Pop maize was the least tolerant while field maize was the most tolerant to broadcast flaming based on the maximum yield reduction obtained with the highest dose propane of 85 $kg\,ha^{-1}$ (Table 6.2).

Field maize flamed broadcast at the 5-leaf stage (V5) was the most tolerant, whereas the 2-leaf (V2) was the most susceptible stage, which had the highest visual crop injury and the largest loss of yield and yield components (Ulloa et al., 2011a). Visual crop injury symptoms included initial whitening and then browning of leaves. Stunting of growth was especially evident when the plants were flamed with higher propane doses (44 and 85 $kg\,ha^{-1}$). Most visual crop injuries, however, were transient as maize plants appeared to be visually recovered within a few weeks. Most growth stages showed higher injury at early evaluation dates compared with later rating dates, suggesting that flamed plants were able to recover over time. Among the yield components, the number of plants m^{-2} and the number of kernels cob^{-1} were the most affected parameters followed by 1000-kernel weight. The loss of crop stands in the plots flamed with higher propane doses occurred due to the complete loss of plant biomass (leaf and stem) that resulted from higher intense levels of heat. Field maize showed a higher tolerance to broadcast flaming at the V5 stage than at the 7-leaf (V7) stage. Flaming particularly at the V7 stage produced plants with thinner and weaker stem than plants flamed at the V5 stage due to a larger loss of leaf area. The thinner stem at the V7 stage combined with the rapid stem elongation might have contributed to stalk breakage resulting in the lowest plant population. The plants with rapid vegetative growth and brittle stalks are often subjected to stalk breakage due to high winds during the field maize-growing season of Nebraska (Elmore and Ferguson, 1999). This may explain the higher yield loss observed with the highest propane dose at the V7 stage. A propane dose of 60 $kg\,ha^{-1}$ was highly efficient in providing 80–90% control of many grasses and broadleaf weeds (Ulloa et al., 2010a, 2010b). A propane dose of 60 $kg\,ha^{-1}$ caused yield losses of 3%, 9%, and 13% for the V5, V7, and V2 growth stages, respectively (Fig. 6.3, Table 6.2). From a practical standpoint,

Table 6.2 List of Broadleaf and Grass Crop Species, their Growth Stages, and Doses of Propane (kg ha^{-1}) that Caused 5% and 10% Yield Reduction [YR_5 and YR_{10} (±SE)]. The Predicted Yield Loss (%) with a Propane Dose of 60 kg ha^{-1} is Represented by PR_{60} for Each Growth Stage

		Propane dose (kg ha^{-1})			
Crops	**Growth stage**	**YR_5 (±SE)**	**YR_{10} (±SE)**	**PR_{60}†**	**YR_{max} (±SE)‡**
Field maize	2-Leaf	23 (2)	37 (2)	13	17 (5)
	5-Leaf	NA*	NA*	3	3 (1)
	7-Leaf	21 (4)	110 (10)	9	11 (3)
Pop maize	2-Leaf	23 (3)	35 (5)	21	45 (4)
	5-Leaf	30 (3)	NA*	8	9 (4)
	7-Leaf	23 (2)	67 (8)	9	16 (2)
Sweet maize	2-Leaf	23 (1)	30 (1)	20	22 (1)
	5-Leaf	25 (2)	28 (8)	11	12 (1)
	7-Leaf	36 (4)	NA*	6	6 (1)
Sorghum	3-Leaf	13 (2)	37 (9)	11	11 (1)
	5-Leaf	72 (17)	NA*	4.6	6 (1)
	7-Leaf	46 (7)	NA*	7	9 (1)
Winter wheat	4-Leaf	11 (4)	16 (4)	50	63 (12)
	3-Tiller	10 (4)	21 (5)	16	21 (3)
	Shoot elongation	3 (2)	8 (4)	25	32 (6)
	Boot stage	5 (1)	9 (2)	52	74 (13)
Soybean	Unfolded cotyledons	55 (2)	70 (2)	6	19 (2)
	Fully unrolled unifoliate	13 (3)	17 (3)	67	96 (17)
	Second trifoliate	21 (2)	29 (4)	30	54 (14)
	Fifth trifoliate	47 (1)	59 (1)	10	30 (6)

*The corresponding yield reduction level (YR_5 or YR_{10}) was not reached with the highest propane dose of 85 kg ha^{-1}.
†A propane dose of 60 kg ha^{-1} was highly effective in providing 80%–90% control of many grasses and broadleaf weeds (Ulloa et al., 2010a, 2010b).
‡The maximum yield reduction (YR_{max}) with the highest propane dose of 85 kg ha^{-1}.
Ulloa et al. (2010c, 2010d, 2010e, 2010f, 2011a, 2011b).

the 3% yield reduction of field maize flamed broadcast at the V5 stage was below the arbitrary assigned acceptable yield reduction of about 5% (e.g. threshold level) and would be acceptable by the organic producers.

Pop maize flamed at the V5 stage was the most tolerant, whereas the V2 was the most susceptible stage to broadcast flaming (Ulloa et al.,

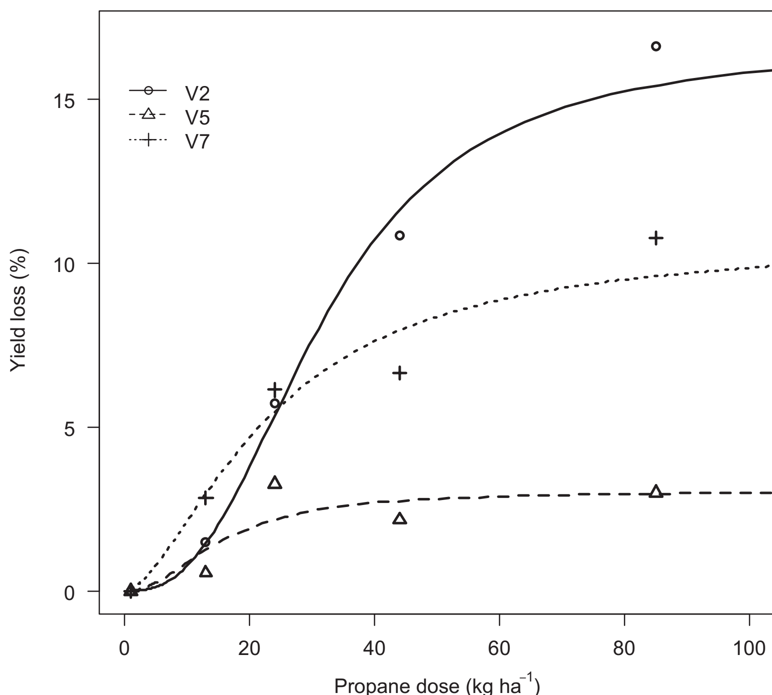


Figure 6.3 Effects of propane dose on field maize yield loss (%) as affected by the growth stage at the time of flaming. The growth stages tested were V2 (2-leaf), V5 (5-leaf), and V7 (7-leaf). (*Ulloa et al. (2011a)*).

2010e). Plants flamed at the V2 stage had the highest yield loss and the lowest yield components. This might be explained by the fact that the ear and tassel tissues are not differentiated at the V2 stage (McWilliams et al., 1999), thus exposing the plants to the stress from heat can result in potentially shorter cobs. In comparison, flaming pop maize plants at later growth stages (e.g. V5 or V7), had less effects on cob size as the ear and tassel tissues start to differentiate at the V5 stage and by the V7 stage, cob and tassel sizes are already predetermined (McWilliams et al., 1999). A propane dose of 60 kg ha^{-1} resulted in an 8%, 9%, and 21% yield reductions at the V5, V7, and V2 stages (Fig. 6.4, Table 6.2), respectively, which would not be acceptable by organic farmers. These yield reductions were the result of the intentional flaming under weed-free conditions in which torches were positioned directly over the crop rows. However, positioning flames below the pop maize canopy would reduce the exposure time to the heat and, therefore, should reduce pop maize yield losses.

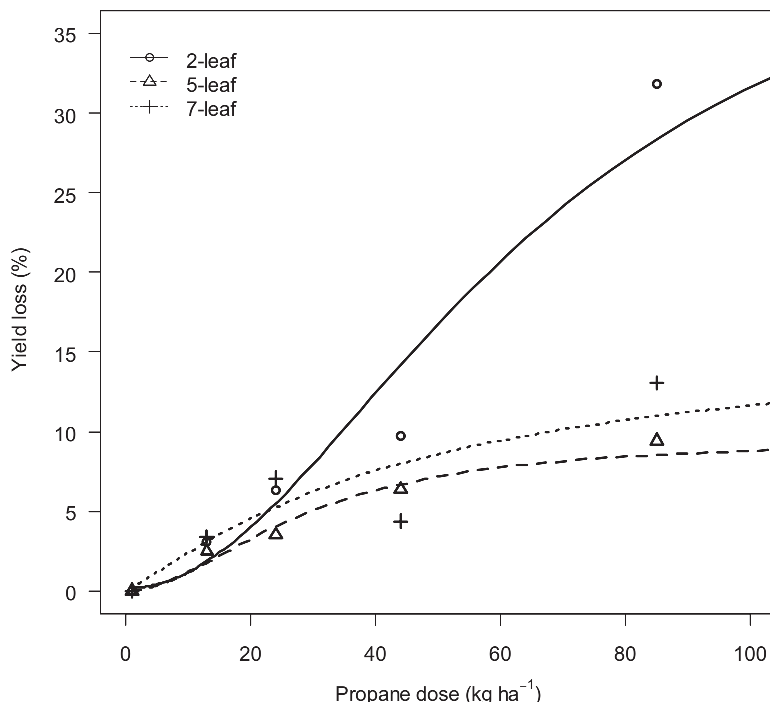


Figure 6.4 Effects of propane dose on pop maize yield loss (%) as affected by the growth stage at the time of flaming. The growth stages tested were V2 (2-leaf), V5 (5-leaf), and V7 (7-leaf). *(Ulloa et al. (2010e))*.

Sweet maize flamed at the V7 stage was the most tolerant, whereas the V2 was the least tolerant stage for broadcast flaming (Ulloa et al., 2010d). Sweet maize flamed at the V7 stage had the least yield loss and the least affected yield components compared to plants flamed at the V5 and V2 stages. The V2 was the most sensitive stage to broadcast flaming resulting in the highest yield loss and the largest effects on yield components. Among the yield components, the number of plants m^{-2} and seeds cob^{-1} were the most affected parameters when flaming was conducted at the V2 and V5 stages. Sweet maize generally starts to accelerate its growth around the V6 to V7 stage (growing point reaches soil surface). This growth acceleration in sweet maize is also coupled with increasing concentration of sugars in cell and stem tissues, which requires more energy to boil water in the cell (Taiz and Zeiger, 2002). A propane dose of 60 kg ha^{-1} caused yield losses of 6%, 11%, and 20% for the V7, V5, and V2 stages, respectively (Fig. 6.5, Table 6.2). From a practical standpoint, the 6% yield reduction of sweet maize flamed broadcast at the V7 stage may not be acceptable by organic growers. However, yield reductions

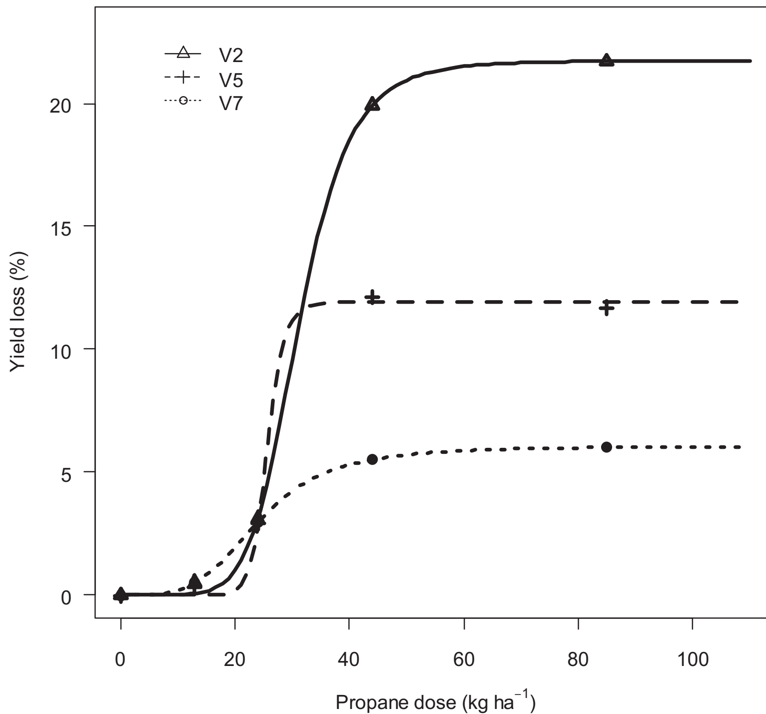


Figure 6.5 Effects of propane dose on sweet maize yield loss (%) as affected by the growth stage at the time of flaming. The growth stages tested were V2 (2-leaf), V5 (5-leaf), and V7 (7-leaf). (*Ulloa et al. (2010d)).*

were the result of the intentional flaming directly over the crop. An alternative might be to direct the flame below the crop canopy to spare foliage from the heat, which could result in lower yield losses (e.g. <5%).

3.2. Tolerance of Sorghum to Flaming

Sorghum plants flamed at the V5 stage was more tolerant, while the V3 stage was more susceptible to broadcast flaming (Ulloa et al., 2011b). Sorghum flamed at the V3 stage resulted in a greater yield loss and lower values of yield components compared to flaming at later growth stages of V5 and V7. Among yield components, the number of heads plant^{-1} and kernels head^{-1}, were most greatly affected by flaming at the V3 stage. Flaming at the V5 and V7 stages did not result in any reduction in heads plant^{-1} or kernels head^{-1}. Heinrich et al. (1983) and Knezevic et al. (1997) reported that the number of heads plant^{-1} and kernels head^{-1} were the components that most significantly and consistently related to the crop yield. The reduction in heads

plant^{-1} following flaming at the V3 stage occurred because some plants were damaged so much that they did not recover to produce heads particularly with greater propane doses (44 and 85 kg ha^{-1}). Similarly, Vanderlip et al. (1977) observed about 5–10% reduction in heads plant^{-1} when flamed at the V3 stage and stand reductions also increased with the severity of flaming treatments. A delay in flowering was also observed following flaming at the early crop growth stages. The head or inflorescence tissue of sorghum is not differentiated by the V3 stage; thus, exposure to any stress at this stage can result in smaller heads with a lower number of kernels due to reduced crop vigor (Vanderlip and Reeves, 1972). In contrast, the head tissues normally differentiate and the potential head size is already predetermined between the V5 and V7 stages (Vanderlip and Reeves, 1972). Flaming at these later growth stages, therefore, likely has little effect on the number of kernels head^{-1}. A propane dose of 60 kg ha^{-1} was highly efficient in providing 80–90% control of many grasses and broadleaf weeds (Ulloa et al., 2010a, 2010b). Sorghum flamed at the V5 stage with a 60 kg ha^{-1} propane resulted in only a 4.6% yield loss (Fig. 6.6, Table 6.2). From a practical point of view, the injury levels and subsequent yield reductions (<5% threshold level) would likely be acceptable by organic sorghum farmers. These yield reductions were the result of treatments and equipment setup intended to cause crop injury. However, it is possible to adjust the angles of torches and direct flames below sorghum canopy, which would result in less yield reduction without affecting the levels of weed control.

3.3. Tolerance of Winter Wheat to Flaming

Winter wheat at the 3T (three tillers) stage was the most tolerant, while the BS (boot stage) was the most susceptible stage to broadcast flaming, which had the highest yield loss and the largest negative effects across all yield components (Ulloa et al., 2010c). Of all yield components, spikes m^{-2} was the most affected by flaming followed by the number of kernels spike^{-1} and 1000-kernel weight. Winter wheat plants flamed at the early vegetative (four leaves-4L) and reproductive stages (BS) exhibited the most sensitivity to broadcast flaming resulting in the highest yield loss. These results support previous findings of Ascard (1994) and Knezevic et al. (2009d), who reported that plant size at flaming time had great influence on plant sensitivity, with small plants being more sensitive than large ones. The higher thermal sensitivity of younger plants is mainly due to their thinner leaves, lower shoot biomass, and less well-protected meristems (Ascard, 1995). In addition, shoot apices of young plants are also more susceptible to heat damage

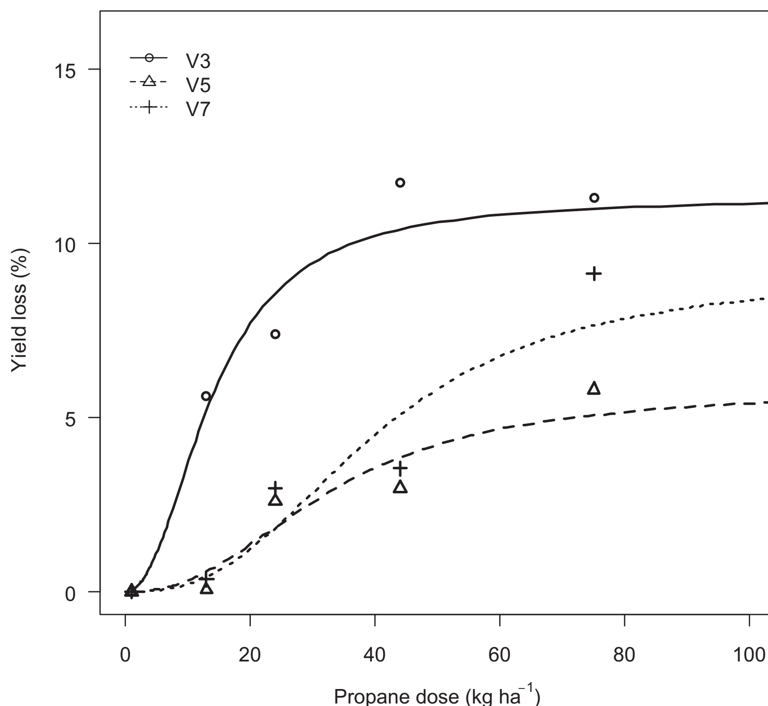


Figure 6.6 Effects of propane dose on sorghum yield loss (%) as affected by the growth stage at the time of flaming. The growth stages tested were V3 (3-leaf), V5 (5-leaf), and V7 (7-leaf). (*Ulloa et al. (2011b)).*

(Ascard et al., 2007). The flag leaf was severely damaged when wheat was flamed at the reproductive stage (BS). Evans et al. (1980) reported that the flag leaf of wheat is the main source of assimilates for the grains. Limited transportation of assimilates to the developing grains due to damaged flag leaf is one of the primary causes for the highest yield reduction at the reproductive stage. Hatfield and Vanderlip (1972) also observed that flaming wheat at the BS caused considerable delay in heading and resulted in severe grain yield reduction. A propane dose of 60 kg ha^{-1} was highly efficient in providing 80–90% control of many grasses and broadleaf weeds (Ulloa et al., 2010a, 2010b). A dose of 60 kg ha^{-1} can cause about 16%, 25%, 50%, and 52% yield reductions for the 3T, SE (shoot elongation), 4 L and BS growth stages, respectively (Fig. 6.7, Table 6.2). Postemergence flaming is, therefore, not recommended in winter wheat due to unacceptable yield loss. However, in situations in which there are high weed populations, flaming could be an option for the farmer to save the crop at the cost of potential loss of yields.

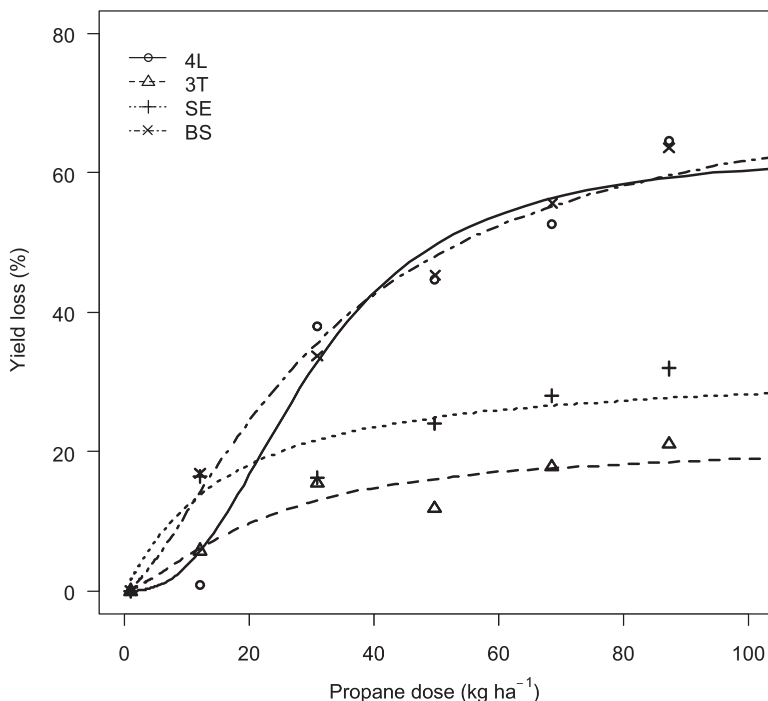
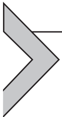


Figure 6.7 Effects of propane dose on winter wheat yield loss (%) as affected by the growth stage at the time of flaming. The growth stages tested were 4 L (4-leaf), 3 T (3-tiller), SE (shoot elongation), and BS (boot stage). (*Ulloa et al. (2010c)*).

3.4. Tolerance of Soybean to Flaming

Soybean at the VC (unfolded cotyledons) stage was the most tolerant, whereas the VU (fully unrolled unifoliate leaves) stage was the most susceptible to broadcast flaming, resulting in the highest visual crop injury and the largest loss of yield and its components (Ulloa et al., 2010f). Soybean flamed at the VU stage was unable to recover over time from the injuries caused by heat; however, plants flamed at other growth stages [e.g. VC, V2 (second trifoliate stage) and V5 (fifth trifoliate stage)] recovered over time to various levels. Unlike maize, where the growing point is protected beneath the soil surface for several weeks, the growing point of soybean is between the cotyledons and moves above the soil surface at emergence. The growing point was partially protected by both cotyledons at the VC stage resulting in the least effect of flaming treatments at this stage. In contrast, the growing point was exposed at the VU stage resulting in severely damaged plants. Consequently, many plants did not get the chance to regrow. Of all yield

components, the number of plants m^{-2} was the most affected by broadcast flaming. A significant number of plants were lost when flaming was conducted at the VU and V2 stages with higher propane doses (e.g. 44 and $85\,kg\,ha^{-1}$). Complete loss of plant biomass (leaf and stem) due to higher heat levels was a reason for the loss of crop stands in the plots flamed with higher propane doses. This provided space for survived plants after flaming at the VC stage to produce more branches $plant^{-1}$ and higher number of pods $plant^{-1}$. A propane dose of $60\,kg\,ha^{-1}$ caused yield losses of 6%, 67%, 30%, and 10% for the VC, VU, V2, and V5 growth stages, respectively (Fig. 6.8, Table 6.2). The 6% yield reduction in soybean flamed at the VC stage was above the arbitrary assigned acceptable yield reduction value of 5%; therefore, it might not be acceptable by organic growers. These yield reductions were the result of the intentional flaming where torches were positioned directly over the crop rows. However, positioning flames below the soybean canopy would reduce the exposure time to the heat and, therefore, should reduce yield losses.



4. ADVANTAGES AND DISADVANTAGES OF FLAMING

Flaming is an attractive weed control option because it leaves no chemical residues in plants, soil, air, or water; produces no drift hazards or herbicide carryover to the next season; and can control herbicide-tolerant or resistant weeds (Nemming, 1994; Wszelaki et al., 2007).

Flame weeding has some major advantages over any herbicide and repeated tillage. Unlike herbicide, flame weeding has no negative impact on the quality of surface or underground water. Flame weeding does not disturb soil structure as repeated mechanical weeding does. It has been reported that repeated cultivation promotes loss of organic matter through the dust particles, and soil erosion induced by wind and heavy rains (Wszelaki et al., 2007). Flame weeding is also significantly less expensive than hand weeding and organic herbicides (Nemming, 1994), and there is likely no chance for weeds to develop resistance to high level of instant heat produced by flaming torches. In comparison with cultivation, flame weeding can be carried out on wet or stony soils, does not disrupt the soil surface, and does not bring buried weed seeds to the soil surface (Ascard et al., 2007; Wszelaki et al., 2007). Flame weeding can significantly reduce the need for hand weeding in organic systems.

The disadvantages of flame weeding when compared to conventional herbicides include higher cost of equipment compared to herbicide

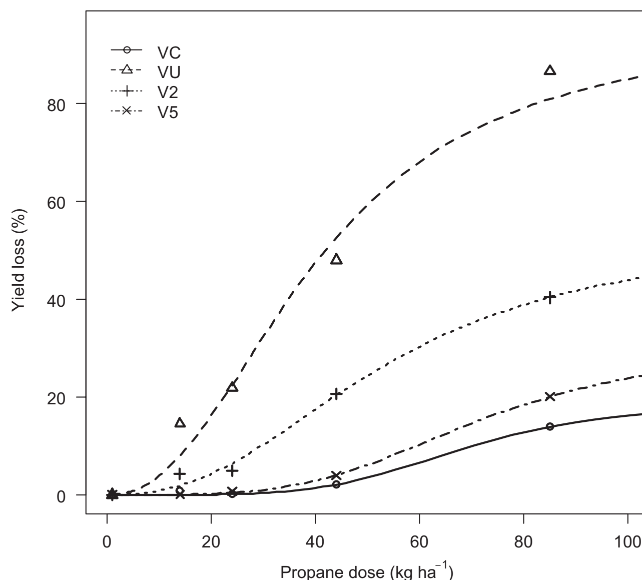
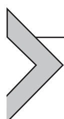


Figure 6.8 Effects of propane dose on soybean yield loss (%) as affected by the growth stage at the time of flaming. The growth stages tested were VC (unfolded cotyledons), VU (fully unrolled unifoliate leaves), V2 (second trifoliate stage), and V5 (fifth trifoliate stage). (*Ulloa et al. (2010f)*).

applicators, lack of selectivity for crop safety, low speed of application due to smaller coverage (e.g. most flamer can treat only 4–8 rows), and lack of residual weed control (Ascard, 1995; Ascard et al., 2007). Flame weeders have about the same field coverage capacity as mechanical cultivators, but are much smaller than the chemical sprayers (Ascard et al., 2007). From a resource and environment point of view, the high energy requirement, the ineffective use of fossil fuels, and the release of carbon emissions in relation to climate change could be seen as disadvantages; however, propane combustion is relatively clean compared to other fossil fuels, for example, diesel (Ascard et al., 2007; Merfield et al., 2009).



5. ENVIRONMENTAL IMPACTS OF FLAMING

The environmental impacts of flaming and chemical control in agriculture on soil, water, air, and energy resources have been described well in the literature (Ascard et al., 2007; Lague et al., 2001). Potential impacts of flame weeding on the soil and environment may include (i) soil compaction resulting from traffic of tractor and flaming implement compared with

chemical control as typically flaming needs to be conducted more often than herbicide application, (ii) the temporary increase in surface temperature of the soil during the flaming treatments (Ascard et al., 2007; Lague et al., 2001), and (iii) the general carbon footprint that can contribute to the overall global warming. However, it has been demonstrated that traffic-induced soil compaction and unwanted heating of the soil surface by the flame are not of significant importance from the environmental standpoint. Flame weeding could impact the air quality to the greater extent than chemical control, as the combustion by-products of propane burning (e.g. CO, CO_2, nitrous and sulfur oxides) are known air pollutants. Such impacts are considered more important from the global warming standpoint than those associated with the use of herbicides such as volatilization and spray drift (Ascard et al., 2007; Lague et al., 2001).

In general, the CO_2 emissions (e.g. carbon footprint) and energy use varied among different methods of weed control (Ulloa et al., 2011a, Table 6.3). A propane dose of 60 kg ha^{-1} provided up to 80–90% control of many annual broadleaf and grass species in Nebraska (Ulloa et al., 2010a, 2010b). Such a propane dose could produce 188.9 kg CO_2 ha^{-1} (180.0 kg CO_2 ha^{-1} from propane combustion plus 8.9 kg CO_2 ha^{-1} from the diesel consumption) from an energy use of 2.90 GJ ha^{-1} (Ulloa et al., 2011a). However, it should be pointed out that the 60 kg ha^{-1} flaming treatment covers 100% of the treatment area. A banded flaming treatment that focuses the heat near the crop row can reduce the required propane dose to as little as 20 kg ha^{-1}. This reduces the energy use and CO_2 emission by approximately 67%; the area not treated by a banded flaming treatment could be controlled with a subsequent cultivation treatment. Such treatment produces 90.8 kg CO_2 ha^{-1} (60.0 kg CO_2 ha^{-1} from propane combustion plus 30.8 kg CO_2 ha^{-1} from the diesel consumption) from an energy use of 1.35 GJ ha^{-1} (Ulloa et al., 2011a).

Manufacturing and applying the recommended dose of glyphosate (active ingredient in Roundup, a commonly used herbicide in Roundup-Ready crops) could produce 98.2 kg CO_2 ha^{-1} (95.9 kg CO_2 ha^{-1} from manufacturing the glyphosate plus 2.3 kg CO_2 ha^{-1} from the diesel consumption) from 0.51 GJ ha^{-1} of energy use. In the case of cultivation, the diesel consumption was 8.2 L ha^{-1}, which produces 21.9 kg CO_2 ha^{-1} from 0.30 GJ ha^{-1} of energy use (Ulloa et al., 2011a).

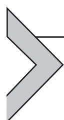
To make a comparison of carbon footprint between the nonchemical and chemical weed control methods, additional studies are needed to determine the overall carbon footprint of production and use of most common

Table 6.3 Comparison of CO_2 Emission and Energy use Among Three Different Alternative Weed Control Methods Available for Conventional and Organic Crop Production Systems

Weed control method	Time (h ha^{-1})	CO_2 emission (kg CO_2 ha^{-1})	Energy (GJ ha^{-1})
Flaming			
Flaming-propane	0.51	180.0	2.78
Flaming-diesel		8.9	0.12
Banded flaming	0.51	60.0	0.93
Spraying			
Spraying-glyphosate	0.13	95.9	0.48
Spraying-diesel		2.3	0.03
Cultivation			
Cultivation–diesel	0.68	21.9	0.30
Total			
Broadcast flaming	0.51	188.9	2.90
Banded flaming*	1.19	90.8	1.35
Spraying	0.13	98.2	0.51
Cultivation	0.68	21.9	0.30

*Banded flaming total includes flaming treatment (20 kg ha^{-1}) plus a cultivation treatment.
Ulloa et al. (2011a).

herbicides currently available in today's agriculture. For example, a typical field in conventional production systems is sprayed with at least several combinations of herbicides applied either preemergence and postemergence, or both, thus contributing to carbon emission. There is a need to determine the carbon footprint associated with the production and application of each of those herbicides. Additional studies are needed to test such a hypothesis.



6. PRACTICAL RECOMMENDATIONS AND FUTURE RESEARCH

Sixty kilograms of propane per hectare was the most effective field application dose to control several annual broadleaf weeds and many grasses. Grass type crops (e.g. field maize, pop maize, sweet maize, and sorghum) are more tolerant to propane flaming than the broadleaf crop (e.g. soybean). Postemergent flaming is not recommended in winter wheat due to a high injury level and unacceptable yield reduction. Maize and sorghum can be safely flamed at their growth stages between VE (emergence) to V10 (10-leaf) stage. Soybean is tolerant to flaming only at the VE–VC stage (emergence-unfolded cotyledon) and at the V4–V5 stage (4–5 trifoliate). It

is not recommended to flame soybean at the VU, V1 (first trifoliate), V2 and V3 (third trifoliate), as these stages are very sensitive to heat and will result in very high crop injury and yield reduction.

Maize and soybean can tolerate a maximum of two postemergence flaming operations per season. Flaming conducted three times in field maize at the V2, V4, and V6 growth stages exhibited >30% injury with as high as 15% yield reduction compared to the weed-free control plots (Nedeljkovic et al., 2011). Flaming soybean three times at the VC, V2, and V5 growth stages resulted in >90% crop injury and as high as 90% yield reduction (Tursun et al., 2011).

With respect to the time of the day, flame weeding is more effective if conducted during the afternoon, but crop injury will be higher, regardless of the propane dose. Thus, flaming could be done around the noon to obtain the maximum weed control with the minimum crop damage (Ulloa et al., 2012).

From the economic standpoint, the costs of a single flaming operation in the USA could be as much as \$16.80 ha^{-1}, without taking into account the costs of the equipment and labor [e.g. the current price of propane (\$0.28 kg^{-1}) in Nebraska was multiplied by the recommended usage dose of 60 kg ha^{-1}; Ulloa et al., 2011a]. Furthermore, Boyd et al. (2006) also reported the total costs of propane flaming with all the associated costs at a \$43.17 ha^{-1}. In their study, they also compared the cost of propane to that of other methods used by organic producers. They concluded that clove oil, an organic herbicide (a mixture of Matran II and Integrate), was the most expensive technique at \$1372 ha^{-1}, which makes its use prohibitive for most situations. This example suggests that propane flaming is much more affordable than many other weed control methods used by organic producers, especially those of hand weeding and organic herbicides.

Flaming has a potential to be used effectively in organic crop production systems of three maize types, sorghum and soybean when conducted properly at the most tolerant growth stage. The observed yield reductions are the result of intentional flaming where torches were positioned directly over the crop. Adjusting the angles of torches and positioning the flames below the crop canopy would reduce the overall plant exposure to the heat and, therefore, should result in lower crop injury and yield reduction. Moreover, some modern precision agriculture techniques such as row detection and weed detection systems could also help in lowering crop injury risks and propane consumption (Van Der Weide et al., 2008).

It is important to mention that propane flaming should not be the only method for nonchemical weed control; however, it could be part of an IWM program. Other measures are still needed to control weeds that emerge later

during the growing season. More research is needed to perhaps develop new flaming equipment and methods, or to examine different positionings of the burners to avoid any significant crop damage and yield reductions. Information from such research would expand flaming options as part of an IWM program for both organic and conventional crop production systems.

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CHAPTER SEVEN

Ridge-Furrow Mulching Systems—An Innovative Technique for Boosting Crop Productivity in Semiarid Rain-Fed Environments

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Contents

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Abstract

Increasing food demands by a growing human population require substantial increases in crop productivity. In rain-fed arid and semiarid areas where the water supply is limited, an increase in the precipitation use efficiency (PUE) is the key to reach this goal. This chapter examines the scientific basis of a ridge-furrow mulching system (RF system) for increasing PUE, and summarizes the effects of this system on crop performance, microclimates, soil attributes, and environmental sustainability. Studies have shown that using crop straw, plastic film, or gravel–sand materials to mulch the soil surface significantly reduces the evaporation of soil moisture, increases water availability to crop plants, and decreases soil erosion caused by wind and water. Plastic mulching increases topsoil temperature during cool spring, promoting plant growth; during hot summer, straw mulching can moderate soil temperature, preventing the topsoil from reaching temperatures that inhibit plant growth. Ridge furrows with plastic mulching on the ridges and crop straw covering the furrows channel water to the furrows, and enhance soil water infiltration and water availability to the crop. Microclimates under mulched ridges and furrows favor soil microbial activity, increase soil biodiversity, and improve environmental benefits. The effectiveness of ridge-furrow systems is reflected in increased crop yields (20–180%) compared with that of the conventional-flat planting. Although more research is required to document physiochemical strengths, technique details and potential drawbacks, and more importantly to define long-term sustainability, we strongly suggest that RF systems are an innovative approach for increasing crop water availability, improving soil productivity, and enhancing food security for arid and semiarid rain-fed areas.

ABBREVIATIONS

RF system ridge-furrow mulching system
CF system conventional-flat-planting system
LF light fraction of soil organic carbon
HF heavy fraction of soil organic carbon
DW dry weight
PUE precipitation use efficiency
MBC microbial biomass carbon
RWCP the percentage of total rainfall channeled to furrows
SOC soil organic carbon
WUE water use efficiency

1. INTRODUCTION

Global food demands are expected to double by 2050 (Tilman et al., 2012); this is driven by the growing human population and the increased need for animal feed. The challenges of meeting the high grain demand and securing global food supply have been increasingly recognized by various levels of policy makers and scientific communities including high-profile features in Science (Godfray et al., 2010) and Nature (Anonymous, 2010). To produce sufficient food and feed, grain crop production must increase by a staggering ≥140%by 2050 according to Food and Agriculture Organization estimates (Bruinsma, 2009). High-yielding technologies suitable for intensive farming systems need to be developed and adopted in a wide range of environments (Siddique et al., 2012). Strategically, a substantial increase in crop yields in developing and in lesser-developed countries will be the key to increasing the availability of food (Chen et al., 2011), reducing the pressure of global grain demand (Godfray et al., 2010), and providing a more equitable food supply across the globe (Bruinsma, 2009). With decreasing availability of well-watered agricultural lands, attempts to reach such high grain production levels need to better use existing crop land with limited water supply, such as those in rain-fed arid and semiarid areas (Fischer and Turner, 1978; Haddad et al., 2010).

In arid and semiarid areas, scarce and unpredictable precipitation and low water availability are the major factors limiting agricultural productivity (Gan et al., 2009; Siddique et al., 2001; Turner, 2004a); this issue has become more serious as global climate change has significant impacts on agricultural systems (Chmielewski et al., 2004; Turner and Meyer, 2011). Inefficient use of scarce water, coupled with drought and heat stress during the cropping season, threatens agricultural sustainability in dryland environments (Siddique et al., 2012; Turner, 2004b). To tackle the problem, new technologies have

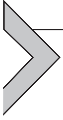
been developed to drastically increase the precipitation use efficiency (PUE) in rain-fed farming systems reliant on precipitation (Turner, 2011). These new technologies include ridge-furrow mulching (Gosar et al., 2010), crop residue retention (Wang et al., 2011b), rainwater harvesting (Li et al., 2006a), no-till farming systems (Gan et al., 2008), strategic supplemental irrigation (Guo et al., 2001), in-site microcatchments (Jia et al., 2006a), and the use of terraces in agroecosystems (Liu et al., 2009).

Among these technologies, ridge-furrow configuration with mulching (RF system) has evolved into a truly innovative system from a conventional-flat-planting system (CF system). The RF system consists of two technical components: mulching and ridge furrowing. Mulching is typically implemented using plastic film, crop straw, animal manure, gravel–sands, rocks, and concrete; these mulches are applied to the field before, during, or shortly after sowing to physically cover the topsoil (Gan et al., 2008; Zhou et al., 2009). The main purpose of mulching is to reduce evaporation and water erosion (Kertesz and Loczy, 1996; Kornecki et al., 2005), improve soil temperature (Wang et al., 2003), and suppress weeds (Hegazi, 2000; Johnson and Fennimore, 2005). Ridge furrowing is where various widths of ridges are built in the field and alternating with corresponding furrows. The soil from furrows is added to the counterpart ridges to channelize rainwater into furrows and to minimize surface water runoff. This system has proved effective in arid to semiarid areas where precipitation is the sole source of water for agricultural production (Zhou et al., 2009). It is most effective in areas where rainfall is sudden, heavy, unpredictable, and infrequent (Huang et al., 2006; Yao and Yin, 1999). Traditional systems often cause up to 85% precipitation losses as runoff (Ren et al., 2009), while runoff can be minimized with RF systems.

The combination of mulching with ridge furrowing is an advanced step in increasing soil water availability. Research studies have found that RF systems increase the penetration of light rain into deep soil (Carter and Miller, 1991; Li and Gong, 2002; Tian et al., 2003), retain surface runoff from heavy rains when ridges are built along the contour (Jia et al., 2006c; Wang et al., 2005; Xie et al., 2005), improve soil water content by reducing water evaporation (Zhang et al., 2007) and prolong the period of moisture available to crops (Li et al., 2000, 2006a). In this system, mulched ridges serve as a runoff surface allowing rainwater to be channeled to furrows and penetrate deeper into the soil, while reducing soil water loss by evaporation from the ridges.

The RF system is being rapidly adopted in field crops for regions where irrigation is not available and soil temperature is low in spring (Liu et al., 2001; Zhang et al., 2005a). We firmly consider that this system is revolutionizing agricultural systems in rain-fed semiarid areas. Some excellent research has

been conducted on this subject, but information from those studies has not been widely summarized in the international scientific literature, thus limiting the application of this technology to other areas. The objectives of this chapter are to (i) evaluate and analyze the scientific basis of RF systems, and (ii) summarize the effects of these systems on crop performance, microclimates, soil water, temperature and biological attributes, and environmental sustainability.



2. VARIETY OF MULCHING SYSTEMS

There are many mulching systems used in the production of field crops. Various materials are applied to the soil surface; these include plastic film, gravel and sand, rock fragments, crop straw, concrete, volcanic ash, paper pellets, and livestock manures (Gan et al., 2008). Gravel–sand and rock fragments represent the most unique materials, whereas plastic film and straw mulching are the most popular. Other types of surface coverings such as a concrete layer, paper pellets, and livestock manures are used in areas where these materials are readily available.

2.1. Plastic Film

Thin plastic film is commonly used as a soil surface mulch for field crop production (Fig. 7.1A) and includes black polyethylene film (Gosar et al., 2010), black perforated film (Gosar and Baričevič, 2011), white transparent plastic film (Subrahmaniyan et al., 2006), and white nontransparent plastic film (Li et al., 1999; Niu et al., 2004). Most plastic films are not readily degradable and have to be removed and destroyed at the end of the cropping season, but new biodegradable plastics are being developed and trialed. The method of applying plastic film varies from crop to crop, but usually the laying of the film and seeding of the crop is accomplished in one operation. Locally adapted seeders are used to form a raised bed (0.3–0.8 m wide; 0.04–0.1 m high), and the crop is sown on the raised ridges or in the furrows depending on the crop species and planting configurations (Li et al., 1999; Niu et al., 1998; Zhou et al., 2011). In maize (*Zea mays* L.) production, for example, seeds are sown in plastic-mulched furrows with holes made in the film to release emerging seedlings when they touch the film. In wheat (*Triticum aestivum* L.), the plastic film covers the furrows (usually shallow) and the crop is sown on the raised covered ridges.

2.2. Ridge Furrow

There are several ridge-furrow planting configurations for field crops (Table 7.1); the most popular ones include (1) alternating ridges and furrows with only ridges being mulched with plastic film (Figs 7.1A and 7.2A);



Figure 7.1 Maize (*Z. mays* L.) planted in ridge-furrow systems with (A) ridges covered with plastic film and (B) ridges and furrows both covered with chopped wheat straw. For color version of this figure, the reader is referred to the online version of this book.

Table 7.1 Different Cover types of Ridge-Furrow Patterns used in Published Research

Ridge-cover type	Furrow-mulch type	Reference
Plastic	Gravel	Li et al. (2000)
Plastic	Pebbles, gravel, fine sand, straw	Li et al. (2001) Li and Gong (2002)
Plastic	None	Liu et al. (2009)
Wheat straw, maize stalks, plastic	Wheat straw, maize stalks	Zhang et al. (2011)
Plastic	Plastic	Jia et al. (2006c) Tian et al. (2003)

(2) bare ridges (compacted to increase channeling) alternating with bare furrows (Fig. 7.1B); (3) ridges covered with plastic film alternating with straw mulched furrows (Fig. 7.2B); (4) ridges and furrows all covered with plastic mulch (Zhou et al., 2009); and (5) alternating mulched rows and bare rows without ridges.

Different ridge-to-furrow ratios have been used in the ridge-furrow system (Li et al., 2007). A higher ridge-to-furrow ratio (2:1 in width) may increase PUE in some crops (Li and Gong, 2002), but optimized ridge-to-furrow ratios depend on multiple factors, including (1) slope of the field, (2) growing-season precipitation, and (3) crop types such as maize, winter wheat, or millet (*Panicum milieaceum* L.). For example, in areas where soil slopes range between 6 and 12°, a ridge:furrow ratio of 0.10:0.30 m was the most effective in preventing runoff and increasing soil water content when rows were along the contour (Li et al., 2008). In wheat production, a normal-sized ridge-furrow unit is 0.6 m of wheat crop sown in furrows along with 0.2 m plastic-covered ridges. In some cases, multiple cropping of wheat/maize/bean (or potato) can be implemented using this system with multiyear use of the plastic or other mulch.

2.3. Crop Straw

Crop straw has been increasingly used as surface mulch in crop production in areas where crop stubbles are not considered as the main source of fuel or fodder for farm families (Huang et al., 2006). In maize and wheat production systems, crop straw is typically chopped and spread evenly on the soil surface followed by no-till direct seeding. Straw is also used such that the seedbed is prepared with shallow rotary tillage followed by sowing, and the straw placed manually on the sown surface (Fig. 7.2B). Some key technical aspects for successful crop straw retention have been identified: (i) the ground should be covered with crop straw from immediately after harvest until sowing the

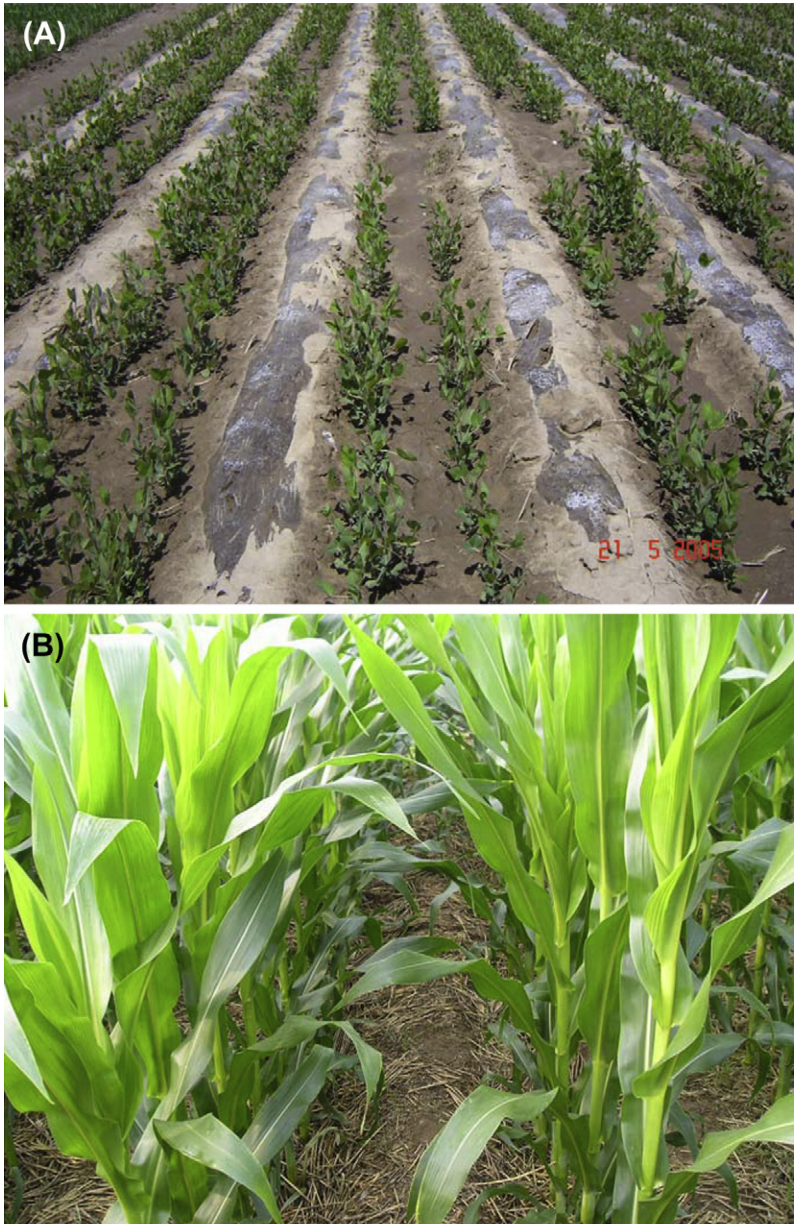


Figure 7.2 (A) A ridge-furrow system with the ridges covered with plastic film and furrows left bare used to grow dry pea (*Pisum sativum* L.); and (B) a ridge-furrow system with both ridges and furrows bare used to grow maize. For color version of this figure, the reader is referred to the online version of this book.

next crop, (ii) the crops should be sown using a no-till drill with minimum soil disturbance, and (iii) weeds should be controlled using herbicides or hand weeding to ensure that there is no cultivation throughout the entire cropping year. In areas where crop straw is used as the main source of animal feed, only a portion of the straw is retained for soil mulch. Thus, the technique is mostly used in areas where crop yields are high and the materials are readily available. However, straw mulching produces inconsistent results with regard to increasing crop yields; some crops may be more sensitive to the straw of other crops due to allelochemical effects (Farooq et al., 2011b).

2.4. Gravel–sand Mulches

For gravel–sand mulching, gravel or rock fragments are applied to the soil surface, and the crop is directly seeded through the gravel–sand covered fields with minimum disturbance (Fig. 7.3). Historically, gravel–sand-covered fields have been used for producing fruit and vegetables, particularly watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] (Fig. 7.3A), but recently, this technique has been used in field crops (Fig. 7.3B). Gravel–sand mulching is usually implemented (or constructed) in fields where a local source of sand and gravel is available. The topsoil layers are loosened by rotary tillage; the soil surface is smoothed and compacted with stone rollers; essential nutrients are applied as nutrient-rich manures and fertilizers; and finally a mix of gravel and sand (0.10–0.15 m) is spread on the soil surface. Crops are usually planted beneath the gravel–sand layers or by making a shallow furrow and seeding the crop in the furrow. This technique is used mostly in the transition zone between arid and semiarid regions where gravel and nearby sand deposits are readily available (Li et al., 2003). With modern devices available for digging, collecting, and transporting gravel and sand, this technique has spread to wider areas, particularly for the production of high-value fruits such as wolfberry (*Lycium barbarum* L.) and jujube or Chinese date [*Ziziphus zizyphus* (L.) H. Karst]. However, construction of such a seedbed is extremely labor intensive, and productivity from a well-constructed gravel–sand-mulched field may only last 8–12 years (Gan et al., 2008), as soil nutrients and organic matter under the mulch deplete rapidly over time, and it is difficult to add nutrients without the gravel mixing with the topsoil.

2.5. Other Mulching Techniques

In addition to plastic, crop straw, and gravel–sand mulches, there are several other mulching techniques. A thin layer of concrete is sometimes used to cover soil surfaces in areas where cement is available nearby at a minimum

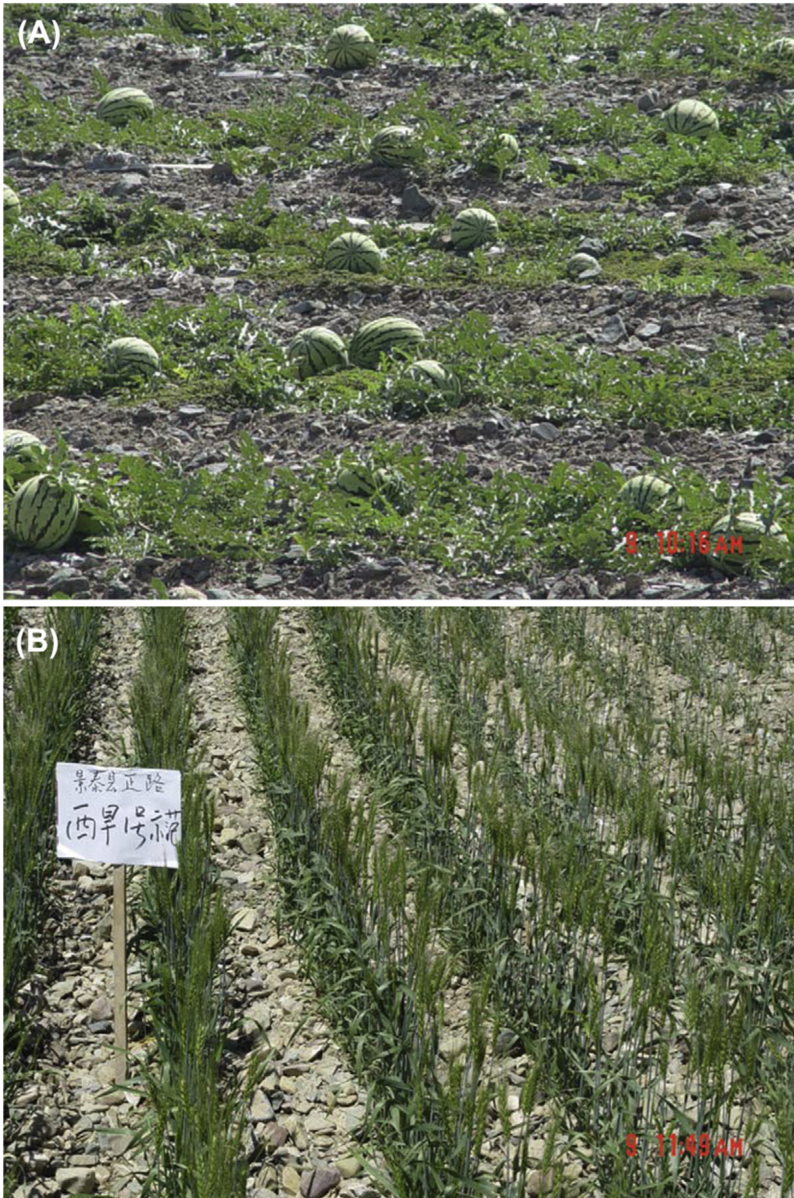
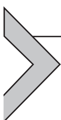


Figure 7.3 Gravel–sand-mulched fields used in the production of (A) watermelon [*C. lanatus* (Thunb)], and (B) spring wheat (*T. aestivum* L.) in an area where the annual precipitation averages 150–230 mm. (For color version of this figure, the reader is referred to the online version of this book).

cost. Usually, the cement is mixed with fine sand in a 30:70 ratio (30% cement/70% sand, v/v), and a thin (0.20–0.30 m) layer of the mixture is applied to the soil surface. Such a layer has similar physical characteristics as that of gravel–sand mulches, and may last several years or a decade after initial construction. However, like gravel–sand mulching, the reduction of soil nutrients and organic matter over time has been a problem with this technique (Gan et al., 2008). Another mulching technique that has been used in a few areas is that of paper recycled into pellets applied to the soil surface as a mulch (Unger, 1975), a form not dispersed by wind and that does not look trashy. However, this is not a popular technique as manufacturing paper pellets is expensive with the current technology.



3. RIDGE-FURROW MULCHING, RAINWATER CONSERVATION, AND SOIL TEMPERATURE

3.1. Rainwater Conservation

Retaining water in the soil and increasing PUE are the keys for crop production in areas where evaporation is greater than precipitation (Turner, 2004b). The configuration of ridges and furrows allows rainwater to be channeled to furrows, and the plastic on the ridges serves as a runoff zone, providing most effective channeling (Fig. 7.4A). Rainwater channeled to the furrows penetrates deeper into the soil profile, reducing soil evaporation, and is readily available to the plants sown in the furrows. Some authors refer to this system as "rainwater harvesting," but RF systems do not create more water, they conserve it, allowing the crop to use precipitation more efficiently and improve PUE. Therefore, we consider the use of the term "rainwater harvesting" as incorrect and recommend that it not be used in future publications. However, mulching does reduce soil evaporation, and the RF system can therefore increase the soil water content. In a rain-simulated laboratory study, Ren et al. (2008) and Wang et al. (2004; 2009) demonstrated that soils with an RF system had consistently higher soil water contents throughout the experimental period than soils with a CF system did. Similarly, under field conditions, ridges covered with plastic film in RF systems resulted in significantly higher soil water contents than did uncovered ridges. In fields with alfalfa (*Medicago sativa* L.), Li et al. (2007) observed that furrows with covered ridges contained 10–50% more soil water than did furrows without covered ridges. In spring maize, the average soil water stored in the upper 1.4-m soil layer in plots with plastic-mulched ridges and furrows was about 40 mm more than that in plots with no mulch (Li et al., 2001). These studies suggest that mulching reduces soil evaporation considerably.

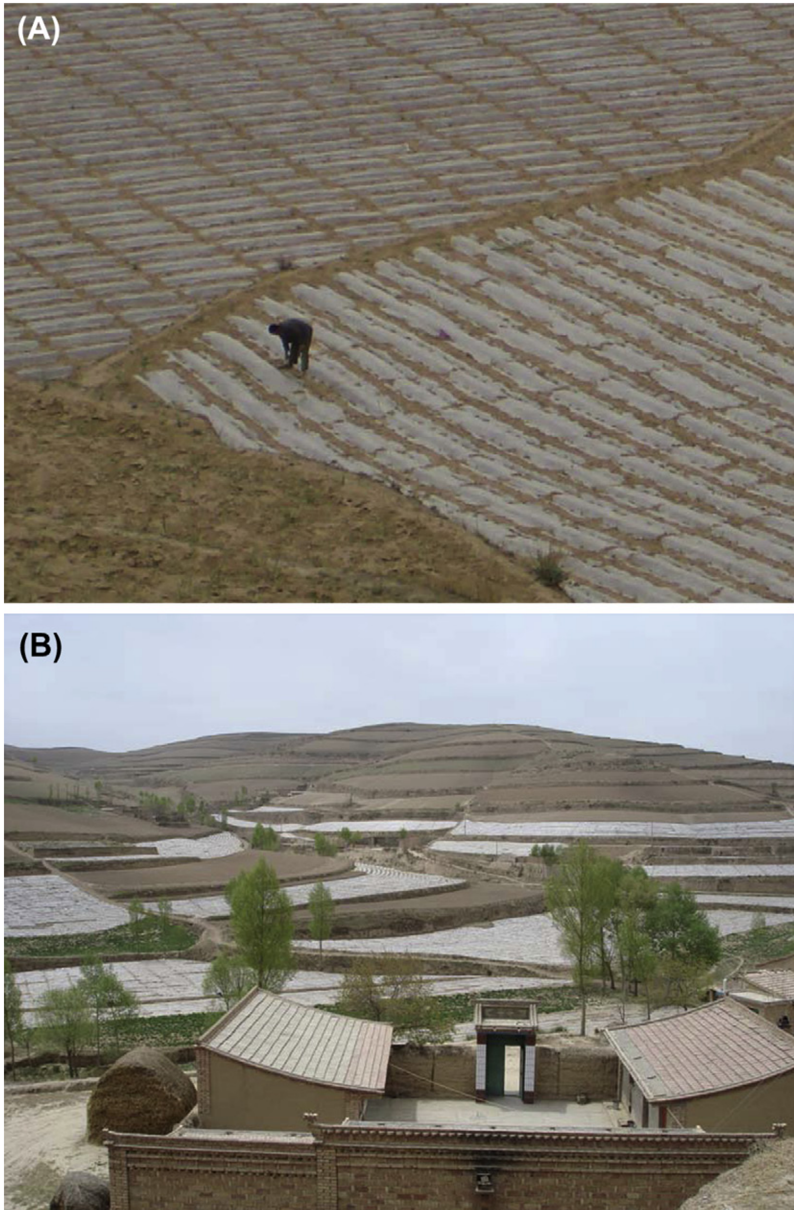


Figure 7.4 Plastic film mulching on sloping areas in Yuzhong County, Gansu Province, China. The upper photograph shows the RF system shortly after planting with the farmer making slits in the plastic film for the maize seedlings to emerge. For color version of this figure, the reader is referred to the online version of this book.

The RF system also helps to increase soil water content deeper into the soil profile. During rainy days, plastic-covered ridges channel rainwater to the furrows from where the accumulated water penetrates deeper into the soil, resulting in more water in the deeper (0.8–1.5 m) soil layers. Water retained in deeper soil layers is less subject to evaporation and is available to crops later in the growing season when the demand is higher and there is little or no rainfall. Also, water retained in deeper soil layers can also benefit crops grown in the following season. In areas with sloping fields where water erosion is usually severe, the RF system can be very effective for water retention (Fig. 7.4B) and water penetration to the soil can be substantial (Li et al., 2007; Ren et al., 2010; Wiyo et al., 1999). For example, after a shower of 27.5 mm, the RF system increased soil water content at a depth of 0.75 m by 42% compared with that in the CF system. In the rainy season (April–September), the amount of soil water penetrating to deeper soil layers was 40–80% greater with the RF system than with the CF system. In some windy areas, rainfall in the hot summer evaporates rapidly with little penetrating the soil in CF systems. In contrast, rainfall retained in the furrows in RF systems often results not only in deeper penetration into the soil but also in lateral movement into the low portion of the ridges, supplying water for crop roots (Ren et al., 2010).

Heavy rainfall (10–20 mm) is considered typical in some semiarid regions (Ren et al., 2010). During hot summer days, evaporation can reach up to 10 mm per day, and with CF systems, the entire rainwater can completely evaporate within 2–3 days. In contrast, with RF systems, rainwater is channeled into and stored in furrows, which then flows laterally back to the ridges for crop use (Fengrui et al., 2000; Li et al., 2000). The amount of water that flows back to ridges will vary with the soil characteristics and the width of ridges. In a study with maize, Li et al. (2007) observed remarkable differences in soil moisture at 1.0 m depth between systems with plastic-mulched ridges 0.30-, 0.45-, and 0.60-m wide; soil water content was the highest with the 0.60-m ridged system, suggesting that more water penetrated into deeper soil layers with wider ridges. However, some other studies did not show differences between ridge widths.

3.2. Rainwater Runoff

With a heavy and intense rainfall, runoff from fields can be substantial with conventional cropping practices, especially on sloping land. With RF systems, the ridges, running along the contour, serve as physical barriers to prevent rainwater from running off, and the rainwater collects in the furrows.

The water channeled to the furrows can rapidly penetrate into deeper soil layers; thus, runoff from fields is minimized. Straw mulch has been found to be better than plastic mulch in preventing/reducing runoff because straw mulch, when coupled with no-till practices, provides a buffer zone, allowing raindrops to penetrate ridges slowly and more effectively, thereby, reducing runoff and sediment load (Table 7.2). To prevent rainwater runoff out of furrows and to reduce sediment loads in the runoff, sowing crops on the contour is required, and no-till practice is suggested. Also, the appropriate type of mulch that suits the frequency and intensity of rainfall is needed to cover the ridges. Most published studies (Table 7.2) show that integrated furrow planting with straw or plastic mulch technology increases soil water infiltration, and prevents runoff and the loss of topsoil from farmland (Li et al., 2007; Ren et al., 2008; Tian et al., 2003; Wang et al., 2011a; Zhou et al., 2009).

In some studies, the term rainwater-harvest ratio was used to describe the amount of rainwater channeled to furrows in proportion to the total rainfall. As indicated earlier, we consider the term rainwater harvesting to be inappropriate in describing rainwater channeling. We suggest the term "Rainwater channeling percentage" (RWCP) be used to more accurately describe the percentage of total rainfall channeled to furrows. Some studies have shown a significant increase in RWCP with RF systems compared with CF systems. Li et al. (2001) measured an average RWCP for plastic-covered ridges in a maize field of 87% (i.e. 87% of total rainfall was collected in the

Table 7.2 Cumulative Runoff, Measured Infiltration and Sediment Load after Intensive Rain (85 mm in 1 h) in Two Rotation Systems with Various Tillage and Crop Straw Retention Practices in Northwest China

Rotation	Treatment	Cumulative runoff (mm)	Total infiltration (mm)	Total sediments (g m^{-2})
Wheat/pea/wheat/**pea**★	NT†	45.9 b★★	39.1 a	28.0 b
	NTS	44.8 b	40.2 a	26.5 b
	T	60.3 a	24.6 b	70.8 a
	TS	50.2 b	34.7 a	32.1 b
Pea/wheat/pea/**wheat**★	NT	62.9 a	22.1 b	32.7 a
	NTS	44.8 c	40.2 a	14.8 c
	T	53.1 b	31.9 b	27.7 ab
	TS	66.3 a	18.7 c	23.8 b

★Crop phase in which the measurements were taken.
†T, conventional tillage; TS, conventional tillage with crop straw incorporated; NT, no-till without crop straw retention; NTS, no-till with crop straw retention.
★★Means in the same column followed by the same letter were not significantly different within a rotation.
Li et al., 2005.

furrows), whereas it was only 7% for uncovered ridges. The value of RWCP is often related to the ridge-to-furrow ratio (Tian et al., 2003; Wang et al., 2005), and an optimal ridge-to-furrow ratio depends on the amount of rainfall (Jia et al., 2006c) and crop species (Ren et al., 2010). For example, Tian et al. (2003) reported that the average RWCP for three mulched ridge treatments (mulched ridges with widths of 0.30, 0.45, and 0.60 m) was 53, 63, and 70%, respectively, compared with the corresponding values of 6, 7, and 10% for three bare-ridge treatments (bare ridges with widths of 0.30, 0.45, and 0.60 m). The proportion of rainfall not harvested was likely lost through surface evaporation, penetration to deeper soil, and runoff. When raised ridges are not covered by plastic film, the total surface area for evaporation is much larger than the area in bare flat plots, leading to rapid evaporation from the raised but uncovered soil surface. Clearly, the value of RWCP will depend on the sampling procedures—depth of sampling of the soil and time after a rainfall event. However, the studies do not identify the determinants influencing the values of RWCP, other than the ridge-to-furrow ratio. More detailed research is required to assess the usefulness of RWCP in reflecting the amount of rainfall that is actually available to the crops.

3.3. Soil Temperature

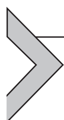
Bare soils typically cool more rapidly and warm more quickly than do covered soils largely due to the albedo effect. Mulching the soil with plastic film prevents water exchange between soil and air, which in turn reduces latent heat flux and the exchange of sensible heat between soil and air. At the seedling stage of crop development, the plant canopy is small, allowing most of the soil surface covered by the plastic film to receive solar energy causing the topsoil to warm. Hence, mulched soil usually warms more quickly than unmulched soil during the day. At night, soil under the plastic cover cools slowly because the plastic film and water underneath the film reduce long-wave radiation. Therefore, mulching with plastic film increases the topsoil temperature early in the cropping season when soil temperature is generally low. In maize, Li et al. (2001) and Moody et al. (1963) found that the soil temperature at 0.10-m depth was significantly higher in RF systems than it was in CF systems early in the growing season (Table 7.3). Plastic-covered ridges are very effective in improving the soil temperature early in the growing season when temperatures are low, as Li et al. (2001) noticed the average soil temperature at a 0.10-m depth in plastic-film-mulched plots increased by 0.5–4.5 °C compared to that in the nonmulched controls. In contrast, mulching with crop straw usually cools the soil in spring, which is

Table 7.3 Summary of the Effects of Covered RF systems Compared to CF system on Soil Temperature

Study site	Study year	Crop	Soil temperature in plastic-covered RF systems compared with CF systems (control)	Reference
Gaolan, China 36°13′N, 103°47′E	1998–1999	Maize	0.98 °C higher at 10 cm depth in the early growing season	Li et al. (2001)
Gaolan, China 35°54′N, 104°06′E	2001–2002	Potato	3–6.8 °C higher in the topsoil in the early growing season	Wang et al. (2005)
Yuzhong, China 36°02′N, 104°25′E	2001–2003	Alfalfa	2 °C higher at 5-cm soil at sowing; 2.2 °C higher at first cut	Jia et al. (2006c)
Blacksburg, USA 37°13′N, 80°25′W	1961–1963	Maize	Warmer while soil was cooling; cooler when soil was hot	Moody et al. (1963)
Yuzhong, China 35° 56′N, 104° 09′E	2010	Switchgrass	Increased soil Temperature by 6 °C above bare soil in April and by up to 10 °C in July	Fan et al. (2012)

disadvantageous to crop establishment as the cool seedbed delays seedling emergence. However, in midsummer, when the air temperature becomes high and heat stress may become an issue for crop growth and development, mulching the soil surface with crop straw reduces the soil temperature significantly compared with bare soil (Chakraborty et al., 2008) or with the soil covered with plastic film (Fan et al., 2012).

However, the effect of ridge furrowing with plastic mulch on soil temperature may gradually diminish as the cropping season progresses for some crop species. In a study on potatoes, Wang et al. (2005) found that early in the season, topsoil temperature in ridges mulched with plastic film was 3.0–6.8 °C higher than that in the uncovered controls, while later in the season, no difference was found, probably because canopy closure minimized any effect from mulches. Similar results were reported by Jia et al. (2006c) in alfalfa that from sowing to the first harvest of alfalfa (4–6 weeks after sowing), the soil temperature at a 50-mm depth in mulched plots was 2.0–2.2 °C higher than that in the unmulched treatments, while soil temperature in mulched treatments was nearly the same as that of the unmulched plots at the end of the growing season. With the closure of the plant canopy, the portion of soil surface exposed to air is minimal, and incoming solar energy is mostly intercepted by crop canopy rather than penetrating the soil.



4. CROP PRODUCTIVITY WITH RIDGE-FURROW MULCHING

4.1. Crop Establishment, Growth, and Development

The biggest advantage of RF systems in field crop production is the promotion of plant establishment and improvement of seedling growth. Many experiments have been conducted to investigate the response of crop establishment to ridge-furrow mulching; the majority of crops showed a positive response (Table 7.4); these crops include maize, spring wheat, potato, and alfalfa. For example, maize seed germination and seedling emergence averaged 2 days earlier and grew more rapidly and vigorously in RF systems than in CF systems in the semiarid Shanxi province of northwest China (Li et al., 2001). Similarly, potato mulched with plastic film emerged 12 days earlier than unmulched control plants (Luan and Wang, 2001), while spring wheat under plastic film emerged 3 (Niu et al., 2004) to 8 days (Li et al., 1999) earlier than did wheat with no mulch. In these experiments, soil moisture under nonmulched treatments was usually lower than that under mulched treatments, but was adequate for seed germination. The increased

Table 7.4 Summary of the Effects of Covered RF systems Compared to CF system on Seedling Establishment and Crop Growth

Study site	Study year	Crop	Crop growth and development in covered RF systems compared to CF systems	Reference
Lanzhou, China 36°06′N, 103°08′E	2001–2002	Wheat	Seedling emergence advanced by an average of 3 days, shoot DW increased by 10–35%	Niu et al. (2004)
Shaanxi, China 34°20′N, 108°04′E	2006–2007	Maize	Seed germination and seedling emergence 1–2 days earlier	Ren et al. (2008)
Gaolan, China 36°13′N, 103°47′E	1998–1999	Maize	Seed germination and seedling emergence 2–3 days earlier; more rapid and vigorous growth	Li et al. (2001)
Gaolan, China 35°54′N, 104°06′E	2001–2002	Potato	Higher biomass during early growing season	Wang et al. (2005)
Yuzhong, China 36°02′N, 104°25′E	2001–2003	Alfalfa	Plants taller; more tillering shoots per plant	Jia et al. (2006a)
Gaolan, China 36°13′N, 103°47′E	2002–2003	Alfalfa	Taller alfalfa plants, more vigorous growth, larger leaves, stronger stems	Li et al. (2007)
Blacksburg, USA 37°13′N, 80°25′W	1961–1963	Maize	Plants at emergence more spindly with narrow leaves	Moody et al. (1963)

seedling emergence was due to the increased temperature of the topsoil (Section 3.3).

However, the magnitude of the effect of RF systems on seedling emergence varies with mulch type and the sowing time of the crop. Some mulch totally covers ridges and/or furrows with little or no exposure of the soil surface, while other types of mulch allow part of the soil surface to be exposed to air so that heat and water may transmit easily and rapidly. Fan et al. (2012) showed that plastic film mulch increased seedling emergence of switchgrass (*Panicum virgatum* L.) sown in spring (April), whereas straw mulch reduced the soil temperature in spring, and no seedlings emerged. The reverse was true when switchgrass was sown in summer (July) with the plastic film reducing the seedling emergence compared with that of straw mulch. In a field experiment conducted in a typical semiarid climate with average annual precipitation <270 mm, Li et al. (2001) found that maize seedlings grown under plastic-covered plots emerged 4–15 days earlier and grew the fastest and were the most vigorous compared with maize seedlings grown under mulches of crop straw, pebbles, gravel, fine sand, or nonmulched control.

Crop growth characteristics including plant height, aboveground dry weight (DW), and root DW are affected by the different mulching systems. In a controlled rainfall experiment, Ren et al. (2008) showed that both plant height and biomass accumulation of maize were significantly ($P < 0.05$) higher in RF systems compared with those in CF systems. Consequently, total DW (shoot + root) in RF systems increased by an average of 41% compared with that in CF systems, largely as a result of the number and size of the cobs (see below for more details). Similar results have been reported by others in wheat and potato (Table 7.4).

The RF system has also been used in the production of some perennial crops such as alfalfa, the most important forage crop cultivated in semiarid areas (Li et al., 2007). Alfalfa plants have deep roots and use more water than annual crops do, particularly after unseasonal rainfall (Blad and Rosenberg, 1976; Li and Gong, 2002; Zhang et al., 2005b). In regions where groundwater is too deep for roots to access the water table, such as the Loess Plateau of China, the channeling of water deeper in the soil profile becomes an important consideration in alfalfa production (Jia et al., 2006c). With conventional production systems, alfalfa typically yields <1.5 tons ha^{-1}, and the yield varies between years and between regions. Yang et al. (2004) studied alfalfa productivity with a high seeding rate and found that the density of alfalfa plants in mulched treatments thinned naturally to an optimum density

with increased height and increased number of lateral shoots per plant. The increased growth of individual alfalfa plants decreased the number of plants per unit area. It is possible that the improved soil–water/temperature balance under mulch promoted self-thinning of alfalfa to an optimum density. Another possible reason is that sparse robust plants often exploit soil water in deeper layers better than do numerous smaller plants with a shallower rooting depth. Interplant competition often is governed by soil moisture-temperature balancing (Berg et al., 2005). In a study on alfalfa, conducted in semiarid northwest China during 2002 and 2003, Li et al. (2007) showed that the date of spring regreening (after the winter) was 15 days earlier for alfalfa grown under plastic-mulched plots than the crop grown in CF plots. The earlier regreening was largely due to increased soil temperature under the plastic mulch in cool spring (Section 3.3).

4.2. Crop Yield and Yield Components

In areas where the growth of crops largely relies on growing-season precipitation such as northwest China, northeast Eurasia, and Siberia steppes, and the northern Great Plains of North America, crop yield is governed by the synchronization of plant growth with rainwater supply. Shortage of water supply at critical stages of plant growth decreases plant growth and lowers crop yield. Mulched RF systems increase the soil water content in the root zone (Sections 3.1 and 3.2). Consequently, field crops grown under RF systems typically have increased yields of 50–100% in drought years, 30–90% in an average-rainfall year, and 10–40% in a wetter-than-normal year, as compared with the same crops grown under CF systems. The increased grain yield with RF systems is typically a combination of increases in various yield components, such as more fertile cobs per plant, increased 1000-seed weight, and more seeds per head (Li et al., 1999; Niu et al., 2004; Ren et al., 2009).

Consistently positive results for crop yield have been reported, but the increases are greater in areas with annual precipitation between 200 and 450 mm. As precipitation increases further, the effect of RF systems on crop yield diminishes. Ren et al. (2009) observed that maize grown under an RF system had 75% higher grain yield when the rainfall was 230 mm, 37% higher yield when rainfall was 340 mm, and there was no difference in yield when rainfall reached 440 mm, when compared with that in CF systems.

Physiologically, the increased crop yield with RF systems is primarily attributable to three factors: (a) *water*—the plastic film mulch directly inhibits evaporation of water from the soil surface, promotes water movement from

deeper soil layers to the topsoil by vapor transfer, and enhances the topsoil water content during critical stages of crop growth; (b) *soil temperatures*—the increased soil temperatures under plastic mulch not only speed up seedling emergence and early growth but also increase the rate of plant development. The latter is crucial especially for maize production in the cooler regions of northwest China where maize grown without plastic film mulching does not reach reproductive development in time to produce a viable cob and seed, but with the plastic film mulch the crop can be planted earlier and emerges earlier so that reproductive development is not compromised; and (c) *lighting conditions*—the ridge-furrowing changes planting configuration in the field. More light is reflected from the raised, white-surfaced ridges to the plant canopy growing in the furrows, allowing more light to penetrate through the bases and sides of the canopy. As a result, mulching with plastic film usually increases the leaf area index of plants, and thus enhances the net photosynthesis of the crop. Although cumulative photosynthetically active radiation (PAR), the amount of light available for photosynthesis during the cropping season, is similar between RF and CF systems, RF planting often enhances the use efficiency of PAR (Liu et al., 2010; Zhang et al., 2011). The improved lighting conditions under ridge-furrow mulching mainly apply in the early stages when the crop canopy is not yet closed, and the effect may diminish as the plants grow and the plant canopy closes.

Fertilizers are typically applied to the crop near the seedlings in both RF and CF systems, but the difference is that with RF systems, the applied fertilizers are under the protection of a plastic cover, so that the potential loss of N fertilizer through volatilization is minimal. Wei et al. (2000) reported that at the same level of manure and fertilizer application, total N and available P in the seeding zone was 17% and 12% more in RF systems than in CF systems during early growth stages. Improved availability of plant nutrients helped increase crop yields. This study suggests that ridge-furrow planting enhances the availability of soil nutrients and improves the effectiveness of fertilization. However, plastic covering may also create a soil environment in which nutrient losses may occur (Sections 5.2 and 5.3).

4.3. Water Use Efficiency

Water use efficiency (WUE) can be defined in various ways (Turner, 1986, 1997). Instantaneous WUE is the rate of photosynthesis divided by the rate of transpiration. In field cropping systems, WUE is defined as grain yield (or biomass) per unit of water use or evapotranspiration. In dryland agricultural systems in which crops are reliant on precipitation, incoming rainfall can

be lost as runoff, deep drainage, and soil evaporation or used by the crop in transpiration (Siddique et al., 2001; Turner, 1997). Therefore, in rain-fed agricultural systems, WUE differs from PUE if the portion of rainwater is lost through runoff or deep drainage. In this chapter, we use PUE when dealing with rain-fed or dryland systems, unless runoff or deep drainage is known.

In arid and semiarid rain-fed areas, increased PUE is generally associated with increased crop yield and water use after flowering (Loss et al., 1997). In northern latitudes or Mediterranean-type environments, strategies and practices to promote early plant growth have been found to increase crop yield and thus PUE (Gan et al., 2009; Siddique et al., 2001; Turner and Asseng, 2005). With RF systems, many studies (Han et al., 2004; Li et al., 2001, 2006b) have shown that the PUE of field crops is substantially greater compared with that of CF systems (Table 7.5). For example, Li et al. (2001) reported that the PUE of maize crops grown in RF systems averaged 62% more than that in CF systems.

Mechanisms responsible for the increased PUE under RF systems are unknown but most likely are attributable to (a) increased crop yield due to early seedling emergence, vigorous plant growth, and more rapid development leading to reproductive success (Table 7.4), (b) improved water and nutrient availability for the crop at critical stages of plant development, and (c) increased leaf area index due to improved light conditions. Some evidence has shown that RF systems prolong the duration of water supply to the crop due to the impermeable barrier, and more available water helps increase the physiologically significant canopy transpiration, thereby enhancing plant physiological processes and thus plant yield; this is in contrast with the CF system where large amounts of soil moisture are lost through nonphysiologically active soil surface evaporation, especially in early development stages when most of the soil surface is exposed to direct radiation and a dry atmosphere.

The magnitude of the effect of RF systems on PUE is often greater when the growing-season precipitation is lower than normal, where mulching helps conserve the limited water available to the crops. In a controlled rainfall study, Ren et al. (2009) found that the PUE of maize in a plastic-covered RF system was 73% greater than that in a CF system when the rainfall was 230 mm, 40% greater when the rainfall was 340 mm, but there was no difference between the RF and CF systems when the rainfall was 440 mm. The advantage of RF systems over CF systems on PUE is greater when water shortage occurs as the mulch reduces soil evaporation losses.

Table 7.5 Summary of the Effects of Covered RF systems Compared with CF systems on the PUE of Different Crops

Study site	Study year	Crop	PUE in RF system compared with CF system	Reference
Gaolan, China 35°54′N, 104°06′E	2001–2002	Potato	Significantly higher ($P < 0.01$)	Wang et al. (2005)
Shaanxi, China 34°20′N, 108°04′E	2006–2007	Maize	Significantly higher ($P < 0.01$); under simulated rainfall (230 and 340 mm), PUE increased by 73% and 40% in 2006; and 77% and 43% in 2007	Ren et al. (2009)
Gaolan, China 36°13′N, 103°47′E	1998–1999	Maize	Significantly higher ($P < 0.01$); 1.9 times greater in 1998; 1.4 times greater in 1999	Li et al. (2001)
Yuzhong, China 36°02′N, 104°25′E	2001–2003	Alfalfa	Significantly higher ($P < 0.01$); PUE was greater in each growing season	Jia et al. (2006c)
Bushland, USA 35°11′N, 102°03′W	1981–1990	Wheat	Significantly higher ($P < 0.01$)	Unger (1992)

Mulched RF systems also help plants to better use low-intensity rains (Willis et al., 1963) by channeling the rainfall into the root zone, and reducing losses by soil evaporation that with unmulched systems can be considerable from frequent small rainfall events. These measures help increase PUE by providing more water for transpiration rather than rainfall being lost as runoff or as soil evaporation.

4.4. Rooting Characteristics

Plants are able to change root morphology in response to soil water, temperature, and nutrients (Clarkson et al., 2000; Gan et al., 2011b). A well-developed root system enables plants to avoid water stress by adjusting root length, DW, and number of root hairs (Sangakkara et al., 1996). Severe water deficit often inhibits root growth, induces irregular root growth (Forde and Lorenzo, 2001), and restricts root functionality (Guo et al., 2001). Soil water availability affects not only the temporal growth pattern of roots during a growing season but also the vertical distribution of root systems in the soil profile (Gan et al., 2011b).

A number of studies have determined the effect of ridge-furrow planting configuration on the root characteristics of field crops (Chakraborty et al., 2008; Gao et al., 2005a; Niu et al., 2004; Rahman et al., 2005; Ren et al., 2010). Nearly all have shown that the RF system improves root development and distribution in soil. Gao et al. (2005a) reported that under drought stress conditions, maize root DW significantly increased in RF systems than in CF systems. The increased root DW was attributed to increased total root length, surface area, and volume of roots (Ren et al., 2010). Similarly, wheat plants grown in a system covered with black polyethylene and rice husks improved root mass significantly (Clarkson et al., 2000). Niu et al. (2004) observed that total root length of spring wheat mulched with plastic film was 23% greater than unmulched wheat, and the effect was consistent across different study years.

Niu et al. (2004) observed that total root DW increased substantially as the crop grew from tillering to soft dough stage for both mulched and unmulched wheat. However, at a given date after seedling emergence, mulched wheat produced significantly more root DW than did unmulched wheat (Fig. 7.5A and B). Both mulched and unmulched wheat increased root DW at a similar rate by peak flowering, but mulched wheat peaked about 10 days earlier than unmulched wheat did. Mulching promoted root growth and development early in the growing season, which resulted in subsequent increases in root DW accumulation during the entire growing

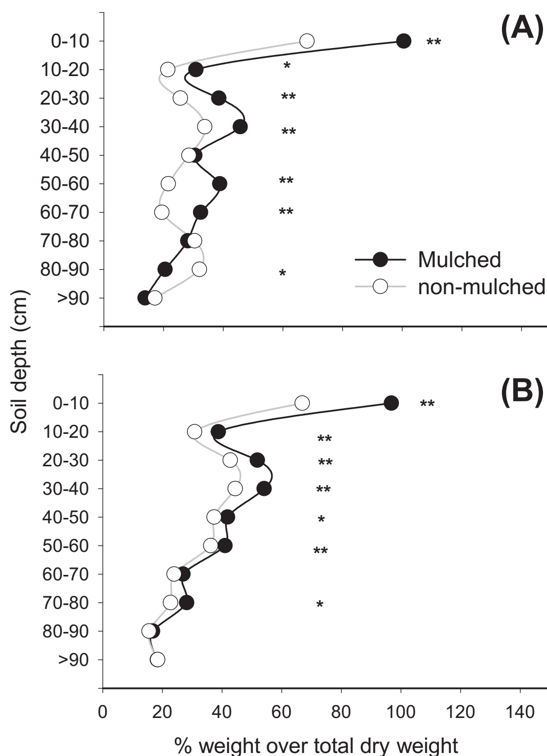
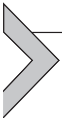


Figure 7.5 Root DW distribution at different soil depths measured 70 days after emergence for spring wheat (*T. aestivum* L.) grown with and without plastic mulch in (A) 2001 and (B) 2002. *, ** Denote significance at a given soil depth between mulched and non-mulched wheat at $P < 0.05$ and $P < 0.01$, respectively. *Niu et al., (2004).*

season and a greater rate of biomass per unit of root. Based on their results, plastic mulch had a marked, positive influence on root DW accumulation partially due to higher soil temperatures (Section 3.3) and improved soil water status (Sections 3.1 and 3.2; Niu et al., 2004).

The positive effect of RF systems on root development is generally related to annual precipitation. For example, Ren et al. (2010) found that rooting systems of maize plants did not differ between RF and CF systems when growing-season rainfall was 440 mm, whereas the two systems differed significantly when growing-season rainfall was 230 mm. Although rainfall collected beneath the furrows benefited root growth when rainfall was limited, it provided no benefit when rainfall was plentiful, probably because it penetrated to depths below the depth of rooting and contributed to deep drainage.

For field crops grown in semiarid environments, increasing rooting depth can dramatically minimize the risk of catastrophic yield loss due to severe drought (Chaves et al., 2003). A study by Jordan et al. (1983) showed that deep-rooting sorghum and wheat increased yields by >20% over shallow-rooted crops. However, such effects of rooting depth on crop yield may diminish in situations where water is not limiting plant growth or where no water is available in deeper soil layers. Therefore, deeper rooting will be advantageous only in situations where soil moisture is available at the depth accessed by deeper roots. If water has not been replenished at this depth, then the strategy of having deeper roots can be risky for the plant, because it means that water and carbon (C) invested in growing deep roots will not contribute to crop yield (Passioura, 1983).



5. EFFECTS OF RIDGE-FURROW MULCHING ON SOIL ATTRIBUTES

5.1. Soil Organic Carbon

Soil organic carbon (SOC) affects the chemical and physical properties of the soil, such as water infiltration ability, moisture holding capacity, nutrient availability, and the biological activity of microorganisms. Soil organic C is a heterogeneous material that can be separated into a light and a heavy fraction (Gregorich and Ellert, 1993; Janzen et al., 1992). The light fraction mainly consists of botanical relics and is more responsible for cropping practices than is the heavy fraction (Biederbeck et al., 1994; Gregorich et al., 1994).

The RF system generally increases aboveground and belowground biomass of field crops and, thus, increases the potential for more organic matter to be returned to the soil. Studies have shown that RF systems increase both light and heavy fractions of SOC (Table 7.6). In a three-year study at Yuzhong, Gansu Province of China, Yu et al. (2007) found that at crop harvest the soils under RF systems significantly increased SOC as compared with CF systems, with the value of SOC increase ranging from 0.69 g kg^{-1} to 1.73 g kg^{-1}. The increased SOC was mainly due to an improvement in the light fraction and less from the heavy fraction. Similarly, in studies by Jia et al. (2006a, 2006b), soils under RF systems increased the light fraction of SOC by 5–49% compared with those under CF system control. The heavy fraction also increased, but the magnitude of increase was smaller than that of the light fraction.

The effect of the RF system on SOC varies with precipitation, as water availability not only affects crop rooting systems and thus the amount of roots returned to the soil mass but also the soil microorganisms responsible

Table 7.6 Effects of RF systems on Soil Carbon (C), Soil Nitrogen (N) and Soil Phosphorus (P) as Compared with Conventional-Flat Systems

Ridge-furrow system	Measurement method*	Effect	Reference
Soil carbon			
Bare ridge-furrow, or only ridges covered with plastic	$K_2Cr_2O_7$ oxidation method	Increased organic C at the end of growing season	Yu et al. (2007)
Plastic-covered or bare ridge-furrow	Elementary Analysen-systeme for soil organic C; chloroform fumigation extraction for soil MBC[†]	Increased total C, MBC,[†] LF,[†] and HF[†]; mulched ridge pattern > bared ridge pattern > control	Jia et al. (2006b)
Bare ridge-furrow or only ridges covered with plastic	$K_2Cr_2O_7$ oxidation method	Increased organic C at the end of the growing season	Jia et al. (2006a)
Plastic-covered ridge-furrow	Outside heating method for organic C	Increased organic C at 230–340 mm precipitation; no differences at 440 mm	Ren et al. (2007)
Soil nitrogen			
Ridge covered with plastic; furrow covered with straw	Continuous flow analytical system for available N	Increased available N in top 0.60 m	Gao et al. (2005b)
Ridge covered with plastic with bare furrow; or bare ridge-furrow	KJ auto analyzer for total N; fluxion injection analyzer for available N	Increased total N under covered RF system Decreased available N under covered RF system	Jia et al. (2006b)
Ridge covered with plastic with bare furrow; or bare ridge furrow	KJ auto analyzer for total N	Increased total N, in the order of covered > bare > control	Jia et al. (2006a)
Plastic-covered ridge and furrow	Alkali N-proliferation for available N	Increased available N	Ren et al. (2007)

Continued

Table 7.6 Effects of RF systems on Soil Carbon (C), Soil Nitrogen (N) and Soil Phosphorus (P) as Compared with Conventional-Flat Systems—cont'd

Ridge-furrow system	Measurement method*	Effect	Reference
Soil phosphorus			
Ridge covered with plastic with bare furrow; or bared ridge-furrow	Vanadate–molybdate-yellow colorimetry for total P; $NaHCO_3$ extraction for available P	Decreased available P during the growing period	Yu et al. (2007)
Ridge-furrow covered by plastic film	$NaHCO_3$ extraction for available P	Plastic-covered RF system increased available P	Subrahmaniyan et al. (2006)
Plastic-covered ridge-furrow; or bare ridge-furrow	Colorimetry for total P; $NaHCO_3$ extraction for available P	Available P: bare > control > covered ridge	Jia et al. (2006a)
Ridge covered with plastic with bare furrow; or bare ridge furrow	$NaHCO_3$ extraction for available P	Available P: covered ridge > bared ridge > control	Jia et al. (2006b)
Plastic-covered ridge furrow	Mo–Sb colorimetry for available P	Plastic-covered ridge increased available P	Ren et al. (2007)

*Details of the methodology are described in the corresponding references.
†MBC, microbial biomass carbon; LF, light fraction of soil carbon; HF, heavy fraction of soil carbon.

for C cycling in the soil. Ren et al. (2007) reported that a plastic-covered RF system in a maize field increased SOC by 0.91 g kg^{-1} with 230 mm precipitation, 0.82 g kg^{-1} with 340-mm precipitation, and did not differ from the nonmulched control treatments with 440 mm of precipitation. In some areas, no-tillage practices have been routinely used in combination with ridge-furrow straw-mulching systems as ridges and furrows, once built, can be used for several years. The combination of crop straw mulching with no-tillage increases SOC more than mulching alone does, especially after a number of years (>5 years). For example, Jiang and Xie (2009) reported that, in a long-term field experiment with rice at the Sichuan province of China where ridge furrow with crop straw mulching had been in place for >5 years, the SOC under the ridges with no-till planting was 48 g kg^{-1}, significantly greater than soil under a paddy-upland system (32 g kg^{-1}) or flooded-flat paddy system (35 g kg^{-1}).

The amount of organic C in the soil depends on the rate of organic matter decomposition and the amount of crop residue returned to the soil (Liang et al., 2010). At any particular time, the total amount of SOC consists of C derived from initial SOC plus C from more recent crop residue inputs, expressed as total SOC = initial SOC + SOC derived from crop residues. The increased SOC in RF systems, compared with that in CF systems, is mainly due to increased SOC derived from crop residues. In cases where only one or two years of crop rotation is practiced, the change in the SOC is very small (Zhou et al., 2009). However, over a longer term, this system may increase the SOC due to increased crop straw and root residues returned to the soil. Therefore, adoption of RF systems has potential for CO_2 mitigation and other environmental cobenefits (Bruce et al., 1999).

5.2. Microbial Biomass Carbon

Microorganisms perform many vital functions in the soil, including converting dead material into plant mineral nutrients. Different soil organisms feed on different organic substrates (Wick et al., 1998). Soil microbial biomass carbon (MBC) is a sensitive indicator of microbial activity and a reflector of soil quality (Benintende et al., 2008).

Studies have shown that the abundance of MBC in soils depends on the configuration of ridges and furrows, their widths and mulching methods. Overall, ridge-furrow patterns increased soil MBC, while the degree of influence varied between experimental locations and crop species. In a study in northern China with alfalfa, Jia et al. (2006b) found that a plastic-mulched RF system with 0.60-m wide ridges alternated with 0.60-m wide furrows

(60:60 ratio) had soil MBC values of 317 mg kg^{-1}, 13% more than the MBC value in the nonmulched RF system with the same ridge-to-furrow ratio, and 22% more than that in the CF system. Increased alfalfa residues under the RF system provided more organic C to support the growth of soil microorganisms. Similarly, a study by Müller et al. (2009) in Germany found that an RF system increased soil MBC by about 5% compared with that in flat plots (215 mg kg^{-1}), confirming that the RF system promotes MBC in the soil.

In a comprehensive field study with maize, Zhou et al. (2009, 2011) designed five treatments: (1) flat-plot sowing with no ridges, furrows or mulching (control), (2) alternating large (0.80 m) and small (0.40 m) ridges fully mulched with plastic film (DRM), (3) on-furrow sowing with plastic mulch applied only on the ridge at an alternating row spacing of 0.60 and 0.40 m (RM), (4) flat-plot sowing with plastic mulch at an alternating row spacing of 0.60 and 0.40 m (NM), and (5) flat-plot sowing with plastic mulch at an alternating row spacing of 0.80 and 0.40 m (WM). They found that plastic film mulching significantly enhanced MBC; the DRM treatment had an MBC value of 633 mg kg^{-1} at the end of the growing season, which was three times the MBC obtained in the control plot. The MBC to SOC ratio was 8.8%, 7.1%, 5.7%, and 5.4% in the DRM, RM, NM, and WM treatments, respectively, significantly greater than the ratio in the control. These results suggest that mulching treatments help increase microbial biomass C more effectively than soil organic C does.

Soil moisture plays an important role in the level of soil MBC, and this is of particular importance in semiarid environments (Li et al., 2004). Smith et al. (1993) showed that there was a strong correlation between soil moisture and MBC. In a study by Zhou et al. (2009), the MBC was the highest in the mulched ridge treatment where topsoil moisture content was also the highest. Additionally, high temperature coupled with favorable soil moisture significantly promoted microbial activity (Kaschuk et al., 2010), and increased plant root growth which, in turn, provided more substrates for microbial biomass (Lynch and Panting, 1982; Yao et al., 2011).

Many reports show that soil MBC is positively correlated with soil organic matter (Smith et al., 1993; Woods and Schuman, 1986). However, increased soil microbial activity may decrease SOC in some cases, because soil microorganisms consume organic C for energy (Song et al., 2003). In a study by Zhou et al. (2011), large increases of soil MBC in fully mulched treatments decreased SOC, probably due to rapid decomposition of soil organic matter by soil microorganisms under conditions of high temperature and favorable soil moisture. The differences between studies in terms

of the effect of mulching on SOC and MBC are likely due to the length of the applied treatments. In the short term (1–3 years), SOC may decrease because microbiological activity requires a good supply of soil C, but in the long term (>3 years), the increased amounts of crop residues under RF systems returned to the soil may help increase the SOC.

5.3. Soil Microorganisms and Biodiversity

Soil microorganisms are critical for chemical transformation in the soil. Most studies have shown that RF systems increase SOC and nutrients (Section 5.1; Table 7.6). As a consequence, soil microorganism community and structure are also affected. In a field study in India, Subrahmaniyan et al. (2006) found under an RF system that the population of soil bacteria, fungi, and actinomycetes increased by 2%, 12% and 12%, respectively, compared with that in a CF system. In the same study, transparent plastic film increased the soil bacteria population more than black or white plastic film did; resulting in more diversified microbial communities than soil under a CF system. Improved light conditions under transparent plastic film may stimulate bacterial microorganisms more favorably than colored films.

Similarly, in a study at Yangling China, Lin et al. (2008) found that plastic-covered RF systems increased the population of soil bacteria, fungi, and actinomycetes by 9%, 83%, and 82%, respectively, compared with a CF system. In these studies, the ratio of microorganisms in the rhizosphere to microorganisms in the bulk soil increased under a covered RF system. In particular, the ratio (rhizosphere/bulk soil) of bacteria, fungi, and actinomycetes increased by 123%, 83%, and 43%, respectively. Using Sabouraud dextrose agar culture medium, Lin and Xue (2009) identified 27 isolates of antagonistic actinomycetes from the RF system, significantly more than the 12 isolates identified from the CF system. The number of antagonistic actinomycetes strains in rhizosphere soil was higher under the RF system (34 strains) than it was in the CF system (21 strains). These studies demonstrate that RF systems change the community structure of soil microorganisms and their functionality, and promote biodiversity in the soil due to changes in soil water content, temperature, organic content, and perhaps crop root secretion caused by these environmental conditions.

5.4. Soil Nitrogen

Total soil nitrogen (TN) and available nitrogen (AN) are important indicators of N cycling activity and soil health. Many studies have determined the impact of mulching systems on soil TN and AN, but the results are

inconsistent (Table 7.6). In some studies, soil TN increased with mulching, while in other studies TN was independent of applied mulching treatments. The inconsistent results are a combination of various factors, including initial soil C and N levels, microbial community and activity, available soil water, and most importantly crop species.

The positive influence of mulching treatments on soil TN has been reported by several researchers (Table 7.6). Jia et al. (2006a, 2006b), working with alfalfa, found that TN increased 10–20 kg ha^{-1}, while Jiang and Xie (2009) reported an increase of 49 kg N ha^{-1} under a plastic-covered RF system compared with the CF system in rice. Also, studies have shown that an RF system with ridges covered by plastic film and furrows covered by crop straw significantly increased soil AN compared to that in the CF system in a wheat-based cropping system (Gao et al., 2005b). In Vridhachalam India, where soil N has traditionally been high, Subrahmaniyan et al. (2006) demonstrated an increase of soil AN from 159 kg N ha^{-1} under a bare RF system to 207 kg N ha^{-1} under a mulched RF system growing peanuts. This increase was probably attributable to an increased nitrogen metabolism by the nitrogen-fixing legume stimulating N cycles in the bacterial community. Müller et al. (2009) in Germany noted that mulching in faba bean (*Vicia faba* L.) fields significantly increased soil microbial biomass N compared with that in a nonmulched control. The amount of increased AN due to mulching varies between studies. In a study with wheat by Gao et al. (2005b) in Shaanxi province China, the average increase in soil AN was 32 kg N ha^{-1} with plastic mulching and 27 kg N ha^{-1} with straw mulching, whereas Ren et al. (2007) found that RF planting did not change soil AN status from that in the control in maize. Apart from the role of legumes in fixing atmospheric nitrogen rather than extracting N for the soil, the increased growth of the crop in the RF system and the increased uptake of nitrogen to enable this growth are likely reasons for the differences in AN among the studies.

Nitrogen status may change in the soil from one form to another (NH_4^+, NO_2^-, NO_3^-) depending on soil microenvironmental conditions. There are inconsistent reports in the literature regarding soil N cycling under RF systems. Jia et al. (2006a, 2006b) with an alfalfa crop showed that nitrate- and nitrite-N in the soil decreased from an average of 16 kg N ha^{-1} under a bare RF system to 12 kg N ha^{-1} in a plastic-covered RF system. In a two-year study on maize, Zhou et al. (2011) found, however, little change in soil TN from sowing to harvest and no differences between mulched and unmulched treatments. Two years of field experimentation is probably

too short a period to see N change. Total soil N content <20 kg N ha^{-1} is usually considered poor soil fertility. In the study of Zhou et al. (2011) on maize, soil TN averaged 8 kg ha^{-1}, indicating low N availability to the crop, and the treatment effect may be masked by low soil N status. Further, in soils fully mulched with plastic film, mineralized N (MN) is usually limited because MN is negatively correlated with microbial biomass C. In such cases, competition for soil N occurs between growing plants and growing microorganisms (Li et al., 2004). Increased plant biomass under RF systems leads to a strong competition between crop plants and microorganisms for MN. Therefore, to gain more biomass from crop plants while avoiding mining soil for N, one must consider adding sufficient amounts of N fertilizer to crops grown under RF systems.

5.5. Soil Phosphorous

Phosphorus is an essential element required by plants, and to satisfy P requirement for crop plants, fertilizer P is generally applied to the soil. One of the main roles of P in living organisms is the transfer of energy, and thereby adequate P availability in soils will stimulate early plant growth and hasten maturity. In natural systems, P cycles in a manner similar to N and other mineral nutrients. Active P, usually in the solid phase, serves as the main source of available P for crops. Active P can be easily released to the water surrounding soil particles. As plants take up P, the concentration of P in solution decreases and more P from the active P pool is released. Crop species and soil conditions such as temperature, moisture, and microbial communities affect the magnitude of release of active P to replenish the soil solution P pool, or provide P nutrient to plants for growth.

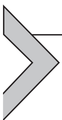
Several studies have been conducted to determine the effect of RF systems on total soil P and available P, but the results have been inconsistent (Table 7.6). In a study with rice, Jiang and Xie (2009) found that an RF system combined with no-till management increased soil total P by 14 kg ha^{-1} compared with that in a CF system. Yu et al. (2007) working with alfalfa reported an increase of total soil P from 12 kg ha^{-1} in conventional-flat plots to 14 kg ha^{-1} in a ridge-furrow system without mulching. Similar findings have been reported by Subrahmaniyan et al. (2006) with peanut. In contrast, Jia et al. (2006a, 2006b), also with alfalfa, found no change in total soil P regardless of ridge-furrow or flat or mulched treatments. Zhou et al. (2011) with maize found little to no difference in total soil P or available P between treatments. Other studies have shown that total P and especially available P decreased with RF systems and in some cases the reduction was very

significant. Yu et al. (2007) showed that a plastic-covered RF system of alfalfa decreased soil available P by 33% (12 vs. 8 kg ha^{-1}) compared with that of a CF system. In studies where soil P decreased with RF systems, soil available water, soil temperature, and crop biomass under the ridge-furrow system were typically higher than those under the control treatment. It is possible that high soil temperature and favorable soil moisture promote microbial activity, but they compete with plants for available P to support their own growth, leading to less soil available P under the covered RF system.

These studies indicate that plastic film mulching generally increases the removal of soil available P by plants due to increased soil temperature and microbial activity, and there is usually a positive correlation between available P and microbial biomass C, suggesting that microorganisms accelerate P cycling in soil. However, P cycling in soil is related to other factors, such as C:P ratio. If the C:P ratio is too high (>300), available P may become low regardless of total P in the soil.

5.6. Relation of Soil Attributes to Plant Biomass

Most studies we reviewed have shown that the use of RF systems can increase soil microbial activity and microbial biomass C, largely stimulated by the higher temperatures and favorable soil moisture conditions. Consequently, crop yields and nutrient uptake are increased, and the total soil C, soil available N and P are decreased. In the short term, the RF systems will increase crop yields significantly compared with CF systems, but sufficient amounts of N- and P-fertilizers should be applied to capture maximum benefits from the system. Evidence has shown that RF systems will renew soil nutrients gradually over time as a larger quantity of crop straw is returned to the soil. More detailed studies involving various residue management options are required to optimize crop productivity while maintaining and improving soil quality in this ecosystem.



6. EFFECTS OF RIDGE-FURROW PATTERN ON ENVIRONMENTS

6.1. Pest Control

Weed control is one of the biggest challenges in agricultural ecosystems (Farooq et al., 2011a). Under field conditions, weeds compete with crop plants for incoming light, soil water and nutrients, and growing space. In some cases, weeds produce allelopathic chemicals into the soil that are toxic to crop plants, leading to reduced crop productivity.

In RF systems, a physical barrier, either in the form of plastic film, crop straw, pebbles, sand gravel, or other material, is applied to the soil surface at the time of sowing. Plastic film mulching completely covers the soil surface physically with only small holes made at spots where crop seedlings will emerge. This mulching system will physically block most weed emergence, and is highly effective in controlling all weed species including grassy and broadleaf weeds.

Mulches other than plastic film may influence microclimate conditions at the soil surface, which will affect the weed spectrum and population either negatively or positively. In a study evaluating precision cultivation effects in a sorghum (*Sorghum bicolor* L.) field in Texas, USA, Allen and Musick (1997) found that herbicide treatments in flat plots controlled 98% of weeds, while the ridge-furrow system combined with adequate tillage activity controlled 95% of weeds, indicating that the RF system provides the same efficacy of weed control by herbicides, but did so with the benefit of minimizing herbicide applications and reducing environmental hazards from chemical inputs. However, in RF systems, there are risks associated with the concentration of herbicide in the furrows that can cause damage to the crop planted in the furrow and/or have a residual impact on a subsequent crop that is sensitive to the chemical. Reducing the amount of herbicide applied can minimize this risk.

In organic and semiorganic farming systems on small-scale farms in China, India, and countries in West Asia and sub-Saharan Africa, weeds are typically controlled through tillage practices or hand weeding. Hand weeding is very labor intensive. Gosar et al. (2010) studied the effect of plastic-covered ridge-furrow pattern on the effectiveness of weed control. Compared with the nonmulching control, plastic mulch reduced the time spent on weed control by 680–830 h ha^{-1}. Later, Gosar and Baričevič (2011) further confirmed, in a two-year study, the time-saving benefit of mulching on weed control. In 2007, hours spent on weed control decreased from 1050 h ha^{-1} in the nonmulched control treatment to less than 190 h ha^{-1} under plastic mulch; in 2008, the corresponding values were 1374 h ha^{-1} down to <170 h ha^{-1}.

Most research of RF-furrow systems focuses on field studies, with a few studies applying this pattern under controlled-environmental conditions. In a greenhouse study with cucumber in the Shaanxi province of China, Chen et al. (2009) found that an RF system increased the rate of root knot nematode (*Meloidogyne* spp.) control by 82% compared with that in a CF system, as the number of susceptible plants decreased from 55 in the control

treatment to seven in the RF treatment. Under plastic film cover, soil temperature increased significantly (Section 3.3), which restricted the activity of some soil enzymes, leading to the death of pathogenic nematodes, fungi, and bacteria in the soil. More detailed research is required to elucidate whether or not increased soil temperatures under plastic mulch would provide similar benefits in controlling other pathogens causing crop diseases.

6.2. Fertilizer Input and Carbon Footprint

Nitrogen fertilizer is the major production input for grain crops other than grain legumes. High use of N fertilizer in crop production results in a large C footprint for grains (Gan et al., 2011c). In some high N-using field crops, the C footprint is a function of the N rate applied to the crop (Gan et al., 2011a). Therefore, reducing N input or increasing N use efficiency through improved farming practices will provide significant environmental benefits.

In a five-year field study on semiarid Vertisols at Solapur India, Maruthi-Sankar et al. (2008) found that RF systems significantly decreased the need of fertilizer N and P during the growing season when compared with CF systems. With a model approach, these authors predicted that to achieve the same target crop yield, the optimal amount of fertilizer N required was averaged at 52 kg ha^{-1} for crops grown in RF plots compared with 177 kg ha^{-1} for crops grown in flat bare plots; for P fertilizer, the corresponding values were 26 kg ha^{-1} in RF plots and 88 kg ha^{-1} in flat bare plots. In northern China, Ren et al. (2009) found that fertilizer nutrient uptake and use efficiency were significantly higher under a plastic-covered RF system than under a CF system; this effect was independent of growing-season precipitation. In their two-year study, fertilizer N use efficiency increased by an average of 43% in year 1 and 38% in year 2. These results imply that using RF systems can significantly improve resource use efficiency and reduce the C footprint of grain products.

6.3. Greenhouse Gas Emissions

The effect of ridge-furrow systems on greenhouse gas emission is poorly understood, as little research has been conducted to determine this effect. It is assumed that the total emission of N_2O, a greenhouse gas with global warming potential 298 times of CO_2 (Gan et al., 2012), is reduced under RF systems as mulches serve as a physical barrier to prevent the gas from emitting to the atmosphere. However, no experimental data have been reported. In a field study with groundnut (*Arachis hypogea* L.) in India, Subrahmaniyan et al. (2006) found that three different kinds of mulch in RF systems

increased CO_2 evolution compared with a bare RF system. In their study, CO_2 evolution under black, white, and transparent plastic film increased 0.06, 0.96, and 0.81 mg of CO_2 100 g^{-1} of soil, respectively, during the cropping season. Increased CO_2 production under mulch may be due to an improved metabolic rate caused by high soil temperature, increased plant respiration, and greater microbiological activity. More research is needed to elucidate the effect.

6.4. Soil and Water Erosion

Soil erosion usually happens in overly disturbed soil systems especially with unfavorable weather conditions. In tropical areas, rainfall and water flow are the main reasons causing soil erosion, whereas on the Loess Plateau of China, water erosion is a great problem. In some arid and semiarid areas, wind can be the cause of soil erosion too. Available information in the literature has well demonstrated that crop residue mulch enhances infiltration and reduces soil erosion, whereas nonerodible materials such as rock are often found to be able to reduce soil erosion by serving as a protective soil cover. Little has been reported regarding the impact of ridge-furrow cultivation on soil erosion, but researchers have realized the importance of the subject.

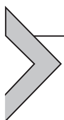
In the arid area of Inner Mongolia, China, where rainfall is typically low and the wind is strong, RF systems significantly decreased soil erosion caused by wind (Liu et al., 2006). Wind velocities ranging from 8 to 24 m s^{-1} were tested under a bare flat system and compared with a ridge-furrow system. Results showed that the average rate of wind erosion under the bare flat system was 130 g m^{-2} min^{-1}, while that under the ridge-furrow system was 20–60% less. Sand transportation rate near soil surface also decreased significantly under the ridge-furrow systems compared with the control. This study suggests that RF systems can be used to prevent or reduce wind-blown soil erosion in arid and semiarid areas.

However, for a given ridge-furrow system, soil erosion in the furrow may be largely determined by sediment and runoff delivery from the ridge area, which, in turn, depends on rainfall intensity, antecedent soil water content, and the plantation practices applied in the system. In a field rainfall simulation experiment on pineapple (*Ananas comosus* L.) cultivation in Hawaii, Wan and El-Swaify (1999) imposed three successive storms: a “dry run” with an intensity of 35 mm h^{-1} on the initially dry soil; a “wet run” with the same intensity on the following day; and a “very wet run” with an intensity of 62 mm h^{-1} that immediately followed the wet run. Their results indicated that plastic mulch substantially accelerated runoff generation and

soil erosion due to its impervious nature. However, the simultaneous presence of plastic mulch and pineapple crowns retarded runoff generation and reduced soil erosion. For all storms, runoff and erosion rates in the plastic-crown plot were 50% less than those of the bare and the crown plots due to the formed microbasins in plastic-crown system that enhanced infiltration. These results show that plastic mulching is an acceptable practice under heavy rainfall conditions, but that crop straw materials must also be used in the furrows and ridges constructed on the contour to prevent water erosion.

Mulching with straw usually prevents the soil surface from sealing and crusting. After rain, fields using these practices usually result in high soil water contents near the soil surface for a longer period of time and greater water infiltration into deeper soil layers. Therefore, mulching can reduce potential water erosion by minimizing runoff. For example, Li et al. (2005) showed that after heavy rain a field of peas mulched with wheat straw increased water infiltration significantly and runoff decreased accordingly. Total sediments decreased by an average of 69% in the pea crop mulched with wheat straw compared with that in a pea field that was not covered with straw.

Mulching with straw also alters the microenvironment near the soil surface. Crop straw retention on the soil surface functions as a physical barrier protecting soil aggregates from the wind. The longer the physical barrier remains on the soil surface, the more effective it is for reducing wind erosion. Additionally, mulching helps create roughness on the soil surface, which traps windblown sediments and dust, thus reducing soil erosion (McFadden et al., 1987). In a simulation experiment, Li et al. (2001) demonstrated that pebble-mulched fields reduced wind erosion rates by >80% compared to nonmulched control. Liu and Li (2005) reported that windblown dust accumulation increased from 0.24 g m^{-2} d^{-1} at 25% soil surface mulching with crop straw to 1.34 g m^{-2} d^{-1} at 100% soil surface mulching. These results illustrate that surface-applied mulches can serve as an effective means to control wind erosion in semiarid environments. In areas where desertification is a concern, soil surface mulching of agricultural land with straw mulch has been recognized as an effective means to reduce the spread of desertification and reclaim land already suffering from desertification.



7. SUGGESTIONS FOR FUTURE RESEARCH

In this chapter, we have mainly focused on the science behind the technique of the ridge-furrow system. We consider that more in-depth research is needed to further evaluate and redefine the feasibility and

sustainability of this technique in the agricultural systems in arid and semi-arid environments. The challenge is to deliver increased crop productivity while improving resource use efficiency and protecting environmental quality. Many topics or subject areas need to be studied in the near future, but our top priorities are outlined below.

7.1. Mechanisms of Improved Water Use Efficiency

There have been consistent reports that RF systems improve PUE, but the mechanisms are unknown. Some studies have demonstrated that, under drought (which often occurs in arid and semiarid areas), hydraulic conductance of plants and their roots is often lower under RF systems than it is under CF systems, but it is unknown whether reduced hydraulic conductance is due to improved water availability in the rooting zone or to less fluctuation in soil temperature. Also, it is important to examine whether plants under RF systems are slower to wilt in response to reduced hydraulic conductance due to osmotic adjustment (Turner and Jones, 1980).

7.2. Plant Physiology and Signaling Systems

In general, crop plants during drought induce a root hormonal signal to shoots, causing stomatal closure and retarding growth without any detectable change in leaf water potential or leaf turgidity (Davies and Zhang, 1991; Davies et al., 1994). Such root-induced signals typically help reduce water loss and allow continued photosynthesis resulting in higher WUE. In the case of ridge-furrow planting, most rainwater is channeled to the furrow where plants are grown—so do the furrows function more or less like microcatchments or basins to make more water available for plant transpiration and/or does the channeling increase the root mass in dry soil and induce signaling under drought?

7.3. Evaluation of the System Sustainability

Double or even triple the grain yields have been reported for crops grown under RF systems compared with CF systems. The substantial increase in crop yield is at the expense of depleting soil C, nitrogen and other nutrients. Most of the studies we reviewed lasted for 2–4 years; none was tested on a long-term basis. Therefore, the sustainability of the RF system is largely undetermined, and no information has been reported in terms of balancing the large amount of grain output and maintenance or improvement of soil fertility. We suggest that some long-term experiments are conducted at various locations with different rainfall intensities and distributions, so that the sustainability of this system can be evaluated at a system level.

7.4. Detailed Relationships between Soil Attributes

Soil MBC is usually correlated with soil organic matter; increasing soil microbial activity decreases SOC, because microbiological activity requires a good supply of soil C. High temperature coupled with favorable soil moisture under plastic mulch typically accelerates decomposition of soil organic matter by soil microorganisms. Loss of SOC means the system is no longer sustainable. However, high crop yield within the RF system will ultimately return more crop residue back to the soil, which should increase the SOC and improve soil fertility in the long run. Therefore, long-term research is needed to determine the impact of RF systems on SOC dynamics and microorganism functionality and biodiversity in the soil.

7.5. Quantification of soil N and Leaching

A considerable amount of fertilizers is required for crops grown under RF systems to achieve high crop yields. Fertilizers are applied under a plastic cover, such that the loss of N through volatilization is minimal. However, under plastic film, with high soil temperature and favorable moisture, we assume that nitrification, denitrification, and N leaching deeper in the soil may increase. Studies are needed to quantify N dynamics and potential leaching losses under various rainfall conditions.

7.6. Greenhouse Gas Emission and Carbon Footprints

Under RF systems, plastic mulches serve as a physical barrier to reduce greenhouse gas emissions to the atmosphere; little research has been conducted to determine the effectiveness of this system in reducing greenhouse gas emission. It is well known that the C footprint of a grain product is a function of total greenhouse gases associated with each kilogram of grain produced. We propose that increased grain yield with RF systems, while minimizing greenhouse gas emissions during crop production, might lower the C footprint of grain crops. On the other hand, increased soil temperature and active microbial activity under plastic cover help deplete soil C rapidly in a short term, while increased crop yields will allow more crop straw and roots to be returned to the soil which may increase soil C in a long term. More research is required to determine and qualify these effects.

7.7. Risk of Pollution

At present, most plastic films used in RF systems are not biologically degradable. Although some biologically degradable materials are being used in trials, their availability and use are still very limited. In view of the potential for

pollution by plastic film, some comprehensive studies at a regional basis are needed to investigate the maximum area that should be covered by crop residue mulching, so that the benefits of using crop straw cover is maximized and the potential environmental impacts of using plastic film is minimized.



8. CONCLUDING REMARKS

The use of mulches such as crop straw, plastic film, sand–gravel, and other materials that physically cover the soil surface insulate the soil surface from the atmosphere. These techniques have been increasingly used in recent years. Among the many benefits, mulching the soil surface significantly reduces soil evaporation and soil erosion caused by wind and water. Plastic mulching increases topsoil temperature during early spring when soil temperatures are low, promoting plant growth, whereas straw mulch moderates soil temperature in the hot summer, preventing the topsoil from reaching temperatures that inhibit plant growth. RF systems, particularly those incorporating plastic on the ridges and straw in the furrows, provide an innovative water-saving strategy in arid and semiarid areas by channeling water into furrows, reducing soil evaporation, and enhancing soil water infiltration deeper into the soil profile, thereby increasing water availability to crop plants. Additionally, mulching ridges and furrows creates a microenvironment that favors soil microbial activity, increases soil biodiversity, and enhances environmental sustainability.

There are some challenges in using the RF system in crop production. In cool and subhumid areas, straw mulch, when applied in excessive amounts, often decreases soil temperatures and delays or even inhibits seedling emergence. Plastic mulching can cause rapid depletion of soil organic matter in the short term, reducing soil quality, and creating visual pollution and an off-season problem of removal and disposal of the film. The high concentration of water in the furrow can lead to deep drainage below the root zone, leaching of mobile nutrients such as nitrogen, or the concentration of herbicides in the crop root zone. These effects are poorly understood. As suggested in Sections 7.1–7.7, more detailed research is required to evaluate and define the optimum use of this system in the development of long-term sustainable systems for rain-fed agriculture. Nevertheless, we believe that RF systems, indigenous to China and India and now spreading around the world, are an important and innovative water-saving tool for increasing crop yields and securing food supply in arid and semiarid regions of the world.

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INDEX

Note: Page numbers followed by "*f*" indicate figures and "*t*" indicate tables.

B

C

D

E

F

O

P

Q

R

S

T

U